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TECHNICAL STUDY 31

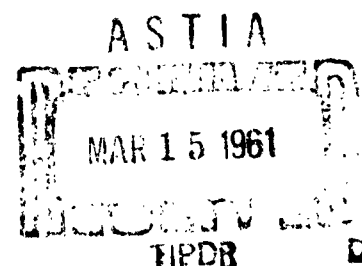
AIR FILTRATION OF MICROBIAL PARTICLES

XEROX

FEBRUARY 1961

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U.S. ARMY CHEMICAL CORPS
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FORT DETRICK



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Kindly substitute these pages for pages 59 and 60 in
U.S. Army Chemical Corps, Biological Laboratories,
Fort Detrick, Technical Study 31, entitled: "Air
Filtration of Microbial Particles" dated February 1961.

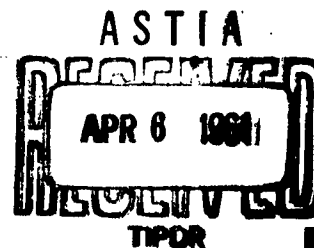


TABLE VI. ROOM CONTAMINATION IN ORGANISMS PER CUBIC FOOT
AT END OF ONE HOUR AND AT STEADY STATE

FILTER EFFICIENCY, %	ORGANISMS BEING GENERATED PER MINUTE		
	1,000	10,000	100,000
30	3.80085* (4.00000)*	38.00852 (40.00000)	380.08520 (400.00000)
60	1.99504 (2.00000)	19.95042 (20.00000)	199.50420 (200.00000)
90	1.33316 (1.33333)	13.33163 (13.33333)	133.31630 (133.33333)
100	1.19994 (1.20000)	11.99946 (12.00000)	119.99460 (120.00000)

Assumptions: 5,000 cubic feet in room; clean at start. Then air changes 10 times per hour through filters. Complete mixing obtained at all times.

* First figure in the body of the table gives concentration in organisms per cubic foot reached at end of one hour. The second figure, in parentheses, gives the equilibrium or steady state concentration. For development of the mathematical solution of this problem, see Appendix D.

in inhabited rooms, including hospital wards or operating rooms. Higher values would be encountered only in areas where some special activity was carried out, such as the handling of soiled linen.^{28,29/}

In the solution to the problem the value given to contamination rates has no effect on the choice of filters, because the equilibrium concentration reached is directly proportional to the contamination rate for any particular filter efficiency. It also is interesting to note that with these parameters the steady or equilibrium state is essentially reached within the first hour and reached a little more rapidly with the more efficient filters. Table VI shows that the concentration figure (equilibrium) for a 60 per cent efficient filter is 2.00000, for a 90 per cent filter 1.33333, and for a 100 per cent filter 1.20000. There is a considerable reduction in organism concentration between 60 and 90 per cent efficient filters, but comparatively little between the 90 and 100 per cent ones. Thus there is considerable benefit in using a relatively efficient (90 per cent) filter as opposed to a relatively inefficient (60 per cent) filter, but very little additional benefit is gained by using a filter which is essentially perfect, or by supplying completely clean air from an extramural source if distribution in the room results in turbulence. However, if flow in the room is streamlined^{22/} (an almost impossible condition to obtain) sterile inlet air will permit the cleanest possible atmosphere. The nearest practical approach to such distribution and to clean air from high-efficiency filters or equivalent devices should be seriously considered for critical areas.

U.S. ARMY CHEMICAL CORPS RESEARCH AND DEVELOPMENT COMMAND
U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Maryland

BL Technical Study 31

AIR FILTRATION OF MICROBIAL PARTICLES

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FOREWORD

This report has been incorporated into the Public Health Monograph series to be published by the Government Printing Office. It is also published in the Biological Laboratories Technical Studies series so that its contents may be more immediately available to Department of Defense personnel.

Variations from the standard format for Technical Studies are attributable to this dual purpose.

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The authors have received helpful suggestions from many associates; however, they wish to make special acknowledgment to the following people of the U.S. Army Chemical Corps, Frederick, Maryland: Dr. Charles R. Phillips and Mr. Floyd Taylor for their assistance in preparing the section on considerations of filtration efficiency requirements for ventilating systems in hospital rooms where contamination is being generated; Mr. Charles M. Dahlgren for completing the sections on preparing the test organisms, and for collecting and culturing media; Mr. J. Bruce Harstad for his comments on viral evaluation methods; Mr. Everett Hanel and Captain John C. Kirsch for review of the manuscript. The authors acknowledge the assistance of Dr. Marvin Harris and Mr. Peter Skaliy of the Public Health Service, Technical Development Laboratories, Savannah, Georgia, in developing the techniques of evaluating air cleaning equipment installed in buildings. The authors also appreciate the assistance of Dr. Leslie Silverman, Harvard University, for his review of the manuscript.

ABSTRACT

Air filtration as a means of removing bacteria is reviewed. Various types of filters have been evaluated, using bacterial organisms as the test simulant. Based on the results of these tests, filters have been placed into four categories: roughing, medium, high, and absolute. Information is also provided on filter evaluation methods, selection of bacterial filters for particular purposes, and methods of installing and decontaminating filters.

DIGEST

Air filtration as a means of removing bacteria is reviewed by the authors, who have been associated for several years with the problem of protecting personnel against disease-producing organisms found in biological laboratories or in hospitals. This study is written specifically to provide architects, engineers, hospital administrators, and research investigators with the benefit of information and experience gained.

Various types of filters have been evaluated, using bacterial organisms aerosolized in particles with diameters of one to five microns as the test simulant. Based on results of these tests, filters have been placed into four categories: roughing (10 to 60 per cent efficient), medium (60 to 90 per cent efficient), high (90 to 99 per cent efficient), and absolute (99.99+ per cent efficient). Other methods of air purification, such as electrostatic precipitation, air washing, ultraviolet light, and incineration are mentioned briefly; however, no attempt has been made to cover in detail the entire field of this technology.

Information is provided on filter evaluation methods, with most of the emphasis on biological techniques. Sections are included on factors to be considered in selecting filters for removal of bacterial particles and on installing and decontaminating filters.

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I. INTRODUCTION

The authors of this monograph have been associated for several years with the problem of protecting personnel against disease-producing organisms found in biological laboratories or in hospitals. Although there are various means of cleaning air, the authors' experiences have been widest in the use of air filtration as a medium for removing bacteria. This monograph is written for the specific purpose of providing to personnel such as architects, engineers, hospital administrators, and research investigators concerned with the confinement and removal of pathogenic biological organisms, the benefit of information and experience gained. Although the writers' interests are mainly biological, certainly this information also is applicable to industries using radioactive material and to others, such as precision instrument manufacturers, who are concerned with the removal of small particles from air. Other methods of air purification, such as electrostatic precipitation and air washing, which remove the particles from the atmosphere, and ultraviolet light and incineration, which destroy the biological organisms, have been used; however, they are only mentioned as a means, and are not discussed here.

In the past, filter manufacturers have been primarily interested in the removal of dust, but only to the extent that the concentration of air-borne particles be reduced to a level that could be tolerated by the people inhaling the air, or by the industrial processes involved. Air cleanliness was considered satisfactory if dust particles were not visible and there were no immediate disagreeable effects such as irritation or odor. It was

felt that any further purification would result in prohibitive filter cost, since the initial and operating costs rise as the efficiency of the filter increases.

In recent years, the subjects of microbiological air pollution, air sanitation, and air cleaning equipment have gained importance. Considerable information is now available concerning the immediate and latent ill effects caused by inhalation and retention of foreign air-borne particles and bacteria.

Present knowledge indicates that those biological particles approximately one to five microns in diameter and those less than 0.1 micron in diameter are of importance in the respiratory transmission of disease. However, larger particles bearing many organisms may be of importance in the infection of open wounds. Therefore, the authors, although fully aware of the difference in importance of various sized particles, suggest that equal emphasis be given to the removal, or inactivation, of biological particles of all sizes from the air used in critical spaces.

The data on the performance of filters, filter media, and other cleaning devices designed for the removal of dust and bacteria from the air sometimes vary considerably from the performance claimed by manufacturers, usually because of differences in the numerous methods of evaluation and variation in test aerosols. Performance of filters to be discussed in this monograph is evaluated on the basis of biological test procedures developed by the authors. It should be pointed out that most published filter evaluations, to the present time, have been based on retention and discoloration as measured by the tests of the American Society of Heating, Refrigeration and

Air Conditioning Engineers (ASHRAE), the Air Filter Institute (AFI), the Chemical Corps' dioctyl-phthalate (DOP) smoke test, and the National Bureau of Standards (NBS) spot test. The results of these test methods at times do not closely correlate with results of test methods using viable bacterial particles.

II. METHODS OF AIR CLEANING

In general, most air cleaning equipment used to remove dust, mist, or fumes from air will also remove some bacteria. There are available many types of air cleaning equipment, which in most cases have been designed for special purposes such as the removal of zinc fumes with particle sizes less than 0.1 micron and particles of chemical sprays such as acid mists (Figure 1). Physical methods involved in air cleaning include (a) gravitational, (b) inertial, (c) filtration, (d) washing, and (e) electrostatic precipitation. In general, most air cleaning equipment will remove from the air the amounts of bacteria in the one- to five-micron particle size range shown in Table I. However, if the bacteria are associated with dust particles to give a particle size greater than five microns, the efficiency will be higher than indicated.

TABLE I. EFFICIENCY RANGE OF DEVICES FOR REMOVING
BIOLOGICAL PARTICLES (1- to 5- μ) FROM AIR

CLEANING DEVICE	BACTERIAL REMOVAL TO BE EXPECTED, %
Absolute Filters	99.99+
High-Efficiency Filters	90 to 99
Medium-Efficiency Filters	60 to 90
Roughing Filters: Fibrous, metallic, oiled, and screen types	10 to 60
Electrostatic Precipitators	80 to 90
Air Washers and Scrubbers (low-pressure-drop type)	20 to 80

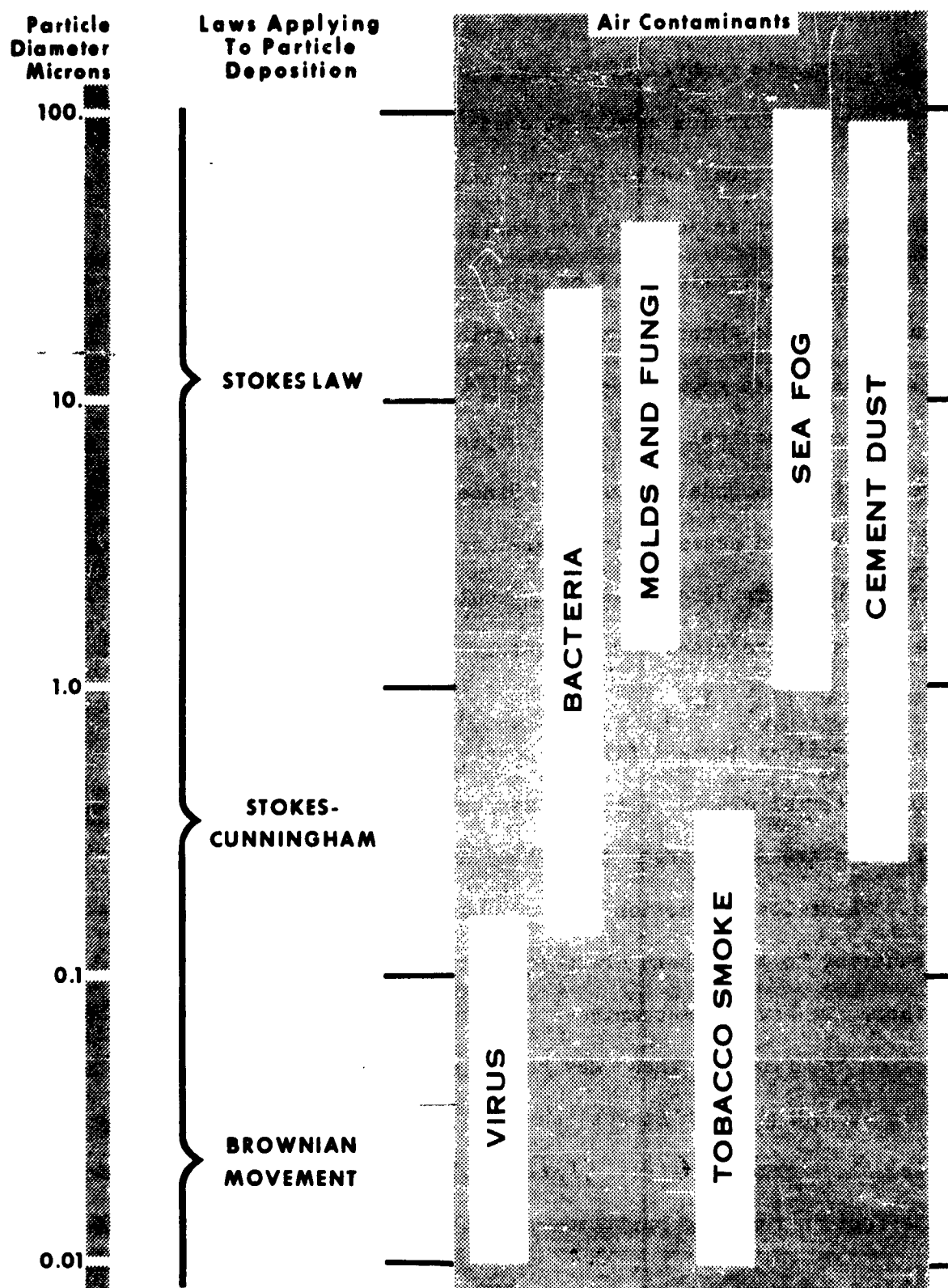


Figure 1. Size Range of Air-Borne Particles.

A. FILTRATION

When complete removal of bacteria or radioactive particles from the air is required, filtration should be used because it is by far the most efficient and practical method of removing the small particles from air, particularly when an approach to sterility is required. The general principles of air filtration will be discussed briefly. For a more detailed discussion of the theory, the publications of La Mer,^{1/*} Langmuir,^{2/} Green,^{3/} and Rodebush^{4/} are recommended.

Very few practical air filters depend upon screening or sieving action to remove the suspended material. Since the interstices of a screening-type filter would necessarily be smaller than the smallest particle to be removed, the resistance to air flow would be high. As the surface becomes loaded, resistance rises and ultimately air flow stops as all of the interstices become plugged.

All practical aerosol filters consist of randomly oriented fibers of various material placed in such a manner that most of the open spaces or interstices are much larger than the diameter of the particles to be removed. The filtering action depends upon the particle's coming in contact and adhering to the fibers or collecting surface.

There are several mechanisms that may cause suspended particles to impact on the fibers. These may be by (a) direct interception, (b) deposition in accordance with Stokes' Law, (c) inertial effect, (d) diffusion, and (e) electrostatic effect. Direct interception and deposition are of less effect in removing particles in filters than the latter three mechanisms.

* See Literature Cited.

In the first mechanism, direct interception is restricted to particles whose centers remain in a given streamline and occurs if the particles are too large to show appreciable Brownian motion and are too small to be appreciably affected by Stokes' Law.

The second mechanism is settling according to Stokes' Law governing rate of fall. If a particle is large enough, it will not coincide with any streamline in the air flow, but will be deposited on the upper surfaces of the filter fibers. The rate of deposition will vary with particle size, concentration, and the total area that the upper surfaces of the fibers project into a horizontal plane. The rate of fall of particles less than 0.3 micron in diameter is so low that this mechanism is probably negligible in removing small particles in filters.

The third mechanism that will cause particles to collect on the filter fibers is the inertial effect. The forces of impaction are generally more effective for collection of particles one micron in diameter and larger. As air flows through a filter, it naturally changes directions to permit flow around the randomly oriented filter fibers. As a result of inertial effects the particles with sufficient mass continue in their original paths and strike the filter fiber despite the change in the path of the air flow. Other micro-aerodynamic forces may also be involved in this method of impaction, but nevertheless tests using bacterial organisms (one- to five-micron diameters) have shown that impaction of particles on fibers is improved as the air velocity is increased through the filter material.^{5/} This is due to the inertial force being directly proportional to the square

of the air velocity and inversely proportional to the radius of curvature of the air stream. It also has been found that decreasing the fiber diameter increases the collection efficiency.^{6/}

The fourth force, diffusion, pertains primarily to small-particulate aerosols. This accounts for the impingement of almost all particles having a diameter less than 0.3 micron. Fine particles of this magnitude diffuse in a manner similar to molecular diffusion, and in the case of passage through a filter are further subject to the laws governing isotropic turbulence.^{7/} Isotropic turbulence occurs when the eddying motion is randomly distributed. Contrary to the action with larger particles, decrease in air velocity through the filter increases the deposition of small particles, since they remain within the filter configuration, resulting in greater opportunities for impingement by the diffusion process. Concomitantly, there is a decrease in deposition of large particles at low velocity because the inertial effect is much less. This is why most commonly used filters have greater collecting efficiency at higher velocities, particularly when the particles are large or in high concentrations.

Still another process by which particles are deposited is by the electrostatic charge that may be present on the filters and particles when air at low humidity passes over the fibers. Certain types of filters may acquire both positive and negative electrostatic charges in various areas of the filter mass. These charges may be strong forces in the removal of particles from an air stream.

It has been shown that, for the most efficient filter, it is desirable to have fibers less than the diameter of the smaller particles to be removed from the air. Small-diameter fibers increase greatly the deposition area of a filter and at the same time increase the free space, thereby reducing resistance to air flow. Small fibers require support to prevent their being packed together and thereby increasing resistance. However, this is not too great a disadvantage, for when small-diameter fibers are used the total contact area is considerably increased and the particles will deposit on the inner fibers of the filters, thereby providing greater loading capacity.

After particles are deposited they are held in place by electrostatic and adhesive forces (i.e. van der Waals and surface tension) between the particles and fibers. Coating fibers with an adhesive such as oil may increase retention. The ability of particles to remain on fibers is more dependent on the nature of the adhesive force than it is on fiber size or particle size until the particle agglomerates get large enough so their cross sections produce sufficient air resistance for detachment.

In considering biological air cleaning, filters will be divided into four categories according to their efficiency and use. Efficiency of all filters considered here is based on their efficacy in removing bacteria one to five microns in diameter from air. The terminology selected should not be considered as identical to that used by manufacturers of air cleaning equipment. These categories are (a) roughing filters, (b) medium-efficiency filters, (c) high-efficiency filters, and (d) absolute filters.

1. Roughing Filters

Roughing filters (Figure 2) are commonly used when large amounts of contamination and debris are in the air. Roughing filters will remove the bulk of large air-borne particles and some will remove 10 to 60 per cent of the bacteria and other particles of a similar size (one- to five-micron diameter); however, most remove less than 50 per cent of one- to five-micron particles. Roughing-type filters may also be used as prefilters for higher-efficiency filters to remove the "sticks and stones" and to reduce "loading" of the more expensive filters.

Two types of roughing filters in general use are the viscous-coated and the dry. Viscous filters are composed of materials such as loosely packed fibers of animal hair, hemp, glass wool, synthetics, or woven metal screens. The fibers are frequently coated with an adhesive substance, usually an oil, which aids in retaining the trapped particles. In some instances these filters are constructed for indefinite use and can be cleaned and re-oiled when the fibers become loaded. Another viscous filter is the metal screen filter, which is automatically cleaned when the belt of metal screen panel moves perpendicularly to the air stream and passes through an oil or water bath at the bottom of the filter unit, where the panels are cleaned and rewetted. The dust collects as sludge in the bath.

The dry type of roughing filter is composed of loosely packed glass fibers, cotton batting, paper, etc. In general it offers more resistance to the passage of air (approximately 0.1 inch water gauge at rated air flows) and has a higher filtration efficiency than the viscous type. However, these filters cannot be recleaned and must be discarded when the resistance to

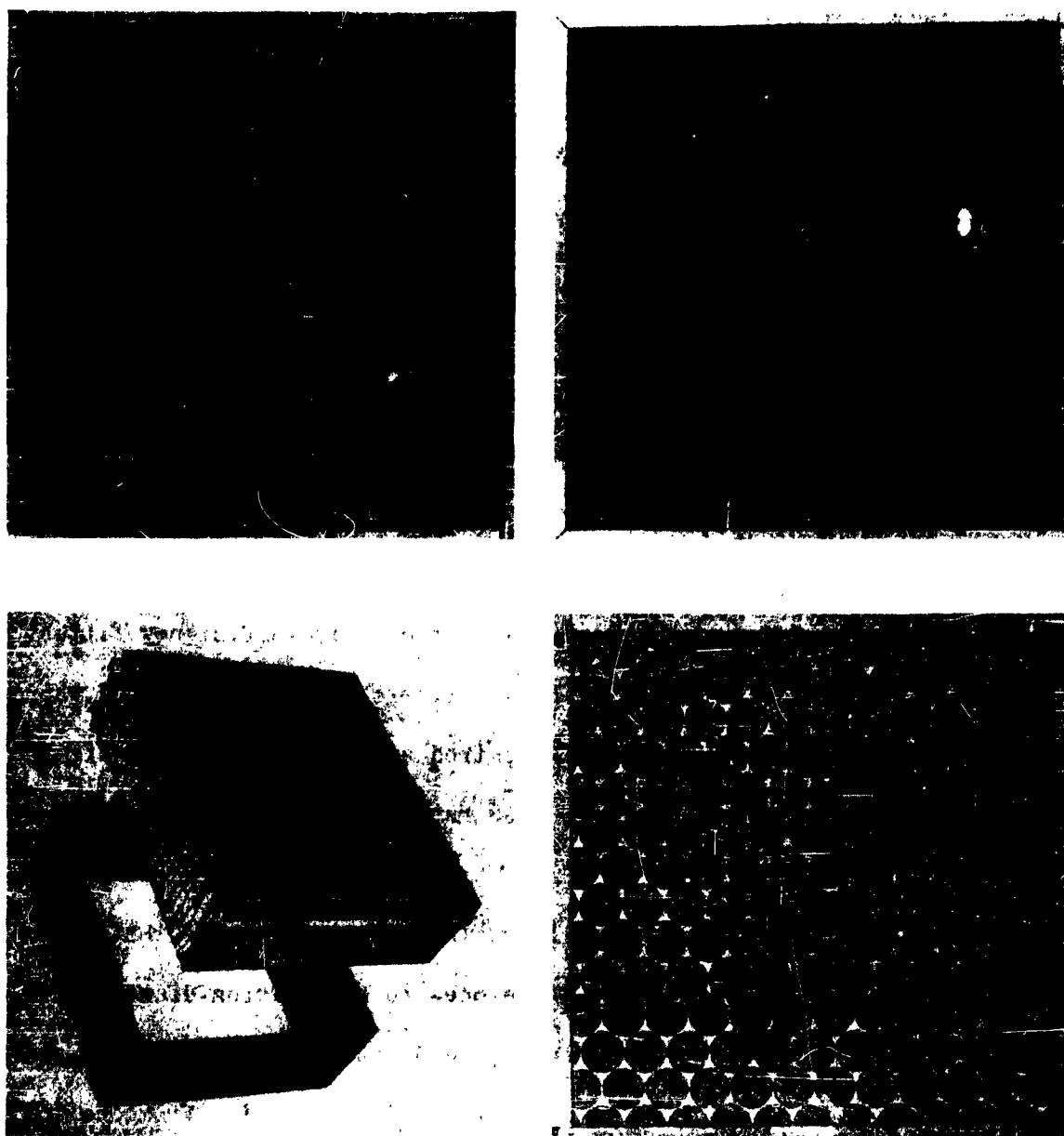


Figure 2. Roughing Filters.

air flow becomes excessive. Filters used in commercial and small home-unit air conditioners are made of loosely packed hair or similar fiber or of woven metal in various forms. This type of filter removes only a small portion of bacteria.

2. Medium-Efficiency Filters

Medium-efficiency filters (Figure 3) are those that remove from the air 60 to 90 per cent of the bacteria and other particles in the one- to five-micron-diameter range. The filter material is usually compressed glass fibers or a good grade of paper fiber. The resistance to air flow is slightly higher than that of roughing filters and increases only slightly when the filters are loaded with dust. When the medium-efficiency filters are loaded, they must be discarded. These filters are generally used where freedom from some specific particle is desired and relatively clean air is required without a large reduction in flow rate.

3. High-Efficiency Filters

High-efficiency biological filters (Figure 4) are those that remove 90 to 99 per cent of all particles in the one- to five-micron-diameter range. The filter media are chiefly glass fibers, good grades of fiber paper, and asbestos fibers, etc., with the diameter of the fiber ranging from one to five microns. Resistance to air flow is higher (approximately 1 inch water gauge at rated air flow) than that of either the roughing or medium-efficiency filters and increases appreciably as the filter load increases. The air flow resistances of these filters may increase fourfold or more before discarding. Another characteristic of these filters is the ability of the medium to carry a heavy bacterial load before the resistance becomes too excessive.

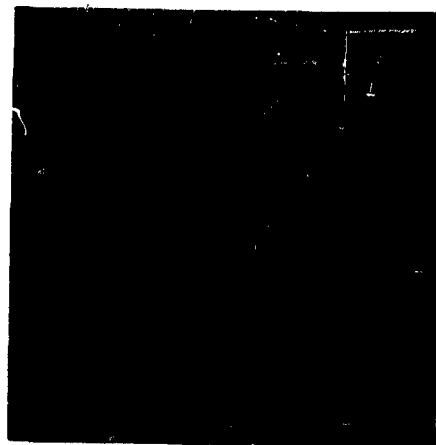
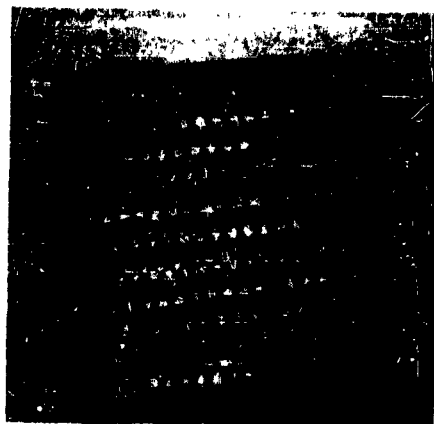


Figure 3. Medium-Efficiency Filters.

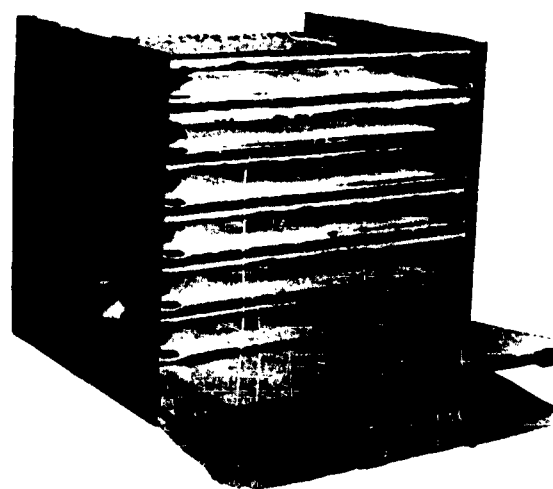
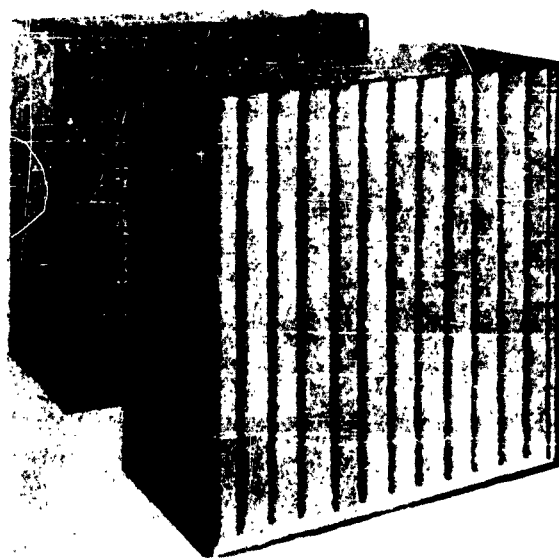
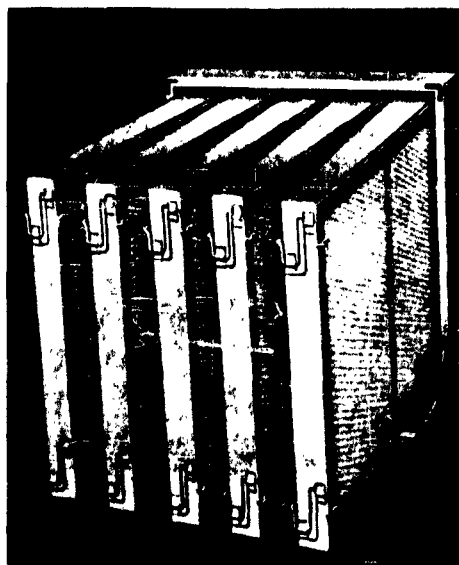


Figure 4. High-Efficiency Filters.

4. Absolute Filters

Filters that are classified as absolute particulate filters (Figure 5) are used to achieve maximum removal of small biological and radioactive particles from air. Absolute filters also are used by the pharmaceutical industry, by electronic manufacturers, etc. to supply particle-free air to certain processes. These filters are more costly, have an initial flow resistance of 1 inch of water, and may be operated to resistances of 4 to 5 inches of water or more before replacement. Absolute filters for the purpose of this monograph will be classified as those that have an efficiency greater than 99.99 per cent for removing bacterial particles having a diameter of one to five microns. Some of the materials used at present in these filters are cellulose asbestos fiber paper, glass and glass-asbestos fiber papers, ceramic fiber paper, compressed glass fibers, and composite beds of glass wool pads. Some of these materials are of recent development and are superior to the older materials because they do not support combustion. Absolute filters have a higher resistance to air flow than the less efficient filters and must be discarded when they become loaded. Face air velocities of absolute filters range between five and seven feet per minute. High air flow capacity in compact size is achieved by pleating the filter to provide increased surface area.

The absolute filter was originally developed by the U.S. Army Chemical Corps for use in gas masks and in building filtration systems for removing bacteria and other particles not removed by charcoal filters. The original absolute filter, unfortunately not fireproof, contained Bolivian or African Blue asbestos, esparto grass, and kraft fibers and was known as

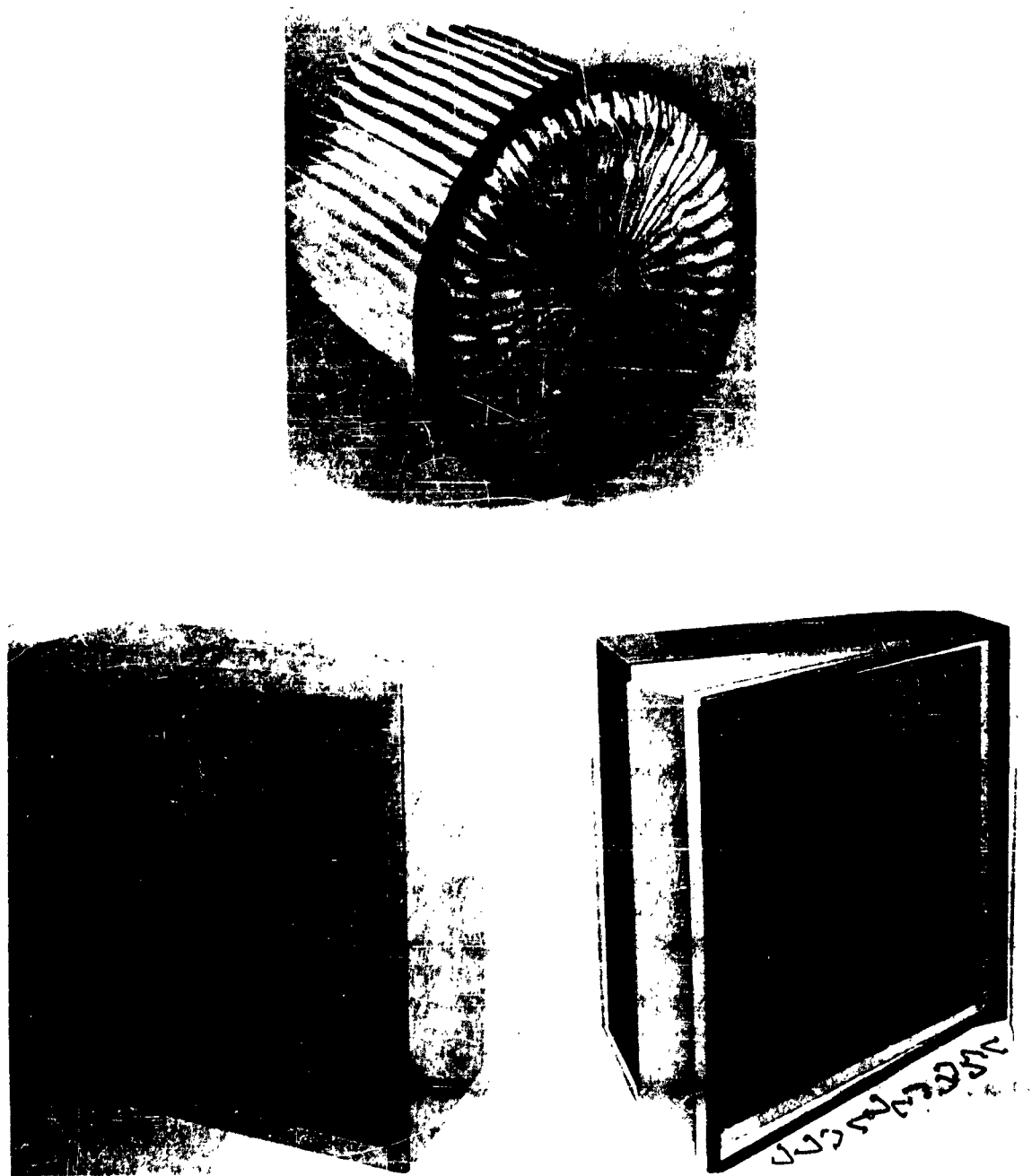


Figure 5. Absolute Filters.

Chemical Corps Type 6 medium. Emphasis by the Atomic Energy Commission on fire resistance has resulted in recent technological developments made on absolute filters. It is now possible to obtain commercially absolute filters designed for fire-resistive (250°F) and high-temperature (1000°F) operation. Fire-resistive designs are constructed with glass or glass-asbestos fiber paper, high-temperature with ceramic fiber medium. One of the glass media for the fire-resistive type is made into filter paper without a binder. Frames, separators, and cements are selected of materials which are incombustible, incorporate fire-suppressing chemicals, or have been impregnated to resist fire spread. In addition to these qualities, absolute filters can be obtained with chemical and high-humidity resistance.

Although absolute filters are excellent for removing all particles down to at least one micron, it is certainly uneconomical to use them alone to remove large quantities of dust and other particles larger than five microns in diameter. The use of roughing, medium, and possibly high-efficiency filters ahead of the more efficient and expensive types places the bulk of the loading on the less expensive filters, extends the life of the absolute filters many-fold, and reduces total operating costs.

B. AIR WASHING AND SCRUBBING

Air washing is another method of air cleaning. However, this method has not been developed extensively for removing bacteria. Air washing is used chiefly for removing dust and other particles from air, although it has been used in some instances for removing bacteria. Spray towers, zig-zag baffles, metal screens, and glass fiber capillary cells constitute some of these types of air cleaning devices. The most efficient air washers are

those in which the suspended matter is impinged on a wet surface and then washed off. Only small amounts of particulate matter are removed by direct contact of the particles with liquid droplets. Scrubbing by itself is not generally a satisfactory method of removing bacteria from air, since the efficiency is usually relatively low. Air washers tested have been found to remove 20 to 80 per cent of the bacteria in the one- to five-micron range. In some instances, where the wash water is recirculated, the actual bacterial count of the air increases because of re-aerosolization of the bacteria that accumulated in the water.

Another air scrubber is the type that uses a hygroscopic solution, usually lithium chloride, to control humidity. These scrubbers generally contain numerous rows of multi-fin coils for temperature control of the absorbent, and have been found to remove approximately 40 to 80 per cent of particles 1.0 to 5.0 microns in diameter, with slightly higher efficiencies for removing larger particles. These scrubbers are an improvement over the standard washers using water, since there is no apparent re-aerosolization of accumulated viable bacteria. The liquids used are generally bacteriostatic, if not bactericidal, and any viable organisms are certainly killed when the liquid is heated to drive the water out of solution. Scrubbers of this type operate automatically, require minimum maintenance, and can serve a dual function, as a humidity control and as pre-filters to protect the final high-efficiency or absolute filters.

C. ELECTROSTATIC PRECIPITATION

Electrostatic precipitation is widely used for reducing air pollution caused by smoke and dust. It has been found satisfactory in many industrial air cleaning applications, removing over 90 per cent of the particles. The equipment operates by passing air through a high-voltage field where the suspended particles are charged and are then collected on electrodes of opposite charge.

The degree of particle removal depends on several factors such as air-flow velocity (which determines the time the particles remain in the field), voltage, the degree of plate loading, and the dielectric properties and size of the particles. Although these units can remove a high percentage of bacteria and dust from air, they may not be as satisfactory as filters where a constant supply of clean air is required. In case of power failure, it would be possible for the contaminated air to pass through the devices, a leakage which could not be tolerated in some situations. Automatic closures could, of course, prevent this occurrence but would increase the cost of the installation. Some units also shut off for a specific period to allow for cleaning, and during such periods no filtration is provided. Electrostatic precipitators that receive maximum maintenance have been shown in laboratory tests to remove or destroy approximately 90 per cent of the microorganisms in the air.^{8/} However, tests of some units under normal operating conditions have shown much lower efficiencies. Operating experience indicates that in many installations, high humidity or small insects or large particles in the entering air cause high-voltage arcing between the elements. This action appears to dislodge deposits from the elements

into the air stream, with resulting recontamination of the air. Arcing due to large particles in the air can be reduced by the use of roughing or medium-efficiency filters ahead of the high-voltage section. Arcing due to high humidity can be corrected only by reducing the humidity of the air before it approaches the electrostatic elements. Arcing becomes so severe in some instances that the elements sustain permanent damage and the devices become inoperable. Under optimum physical conditions, and with satisfactory maintenance, electrostatic precipitators could be used in place of medium-efficiency filters. Without maximum maintenance of electrostatic precipitators, a false sense of security may be obtained.

D. AIR INCINERATION

Although filtration can be applied to most situations, there are certain conditions that require the use of air incineration rather than mechanical filtration. In such situations, the organisms are not removed from the air, but instead are killed by heat. This distinction is important, for when high concentrations of pathogenic microbial aerosols are created for the study of infectious diseases, they should be discharged through incinerators.

When there is a chance of the escape of high concentrations of pathogens to the atmosphere where people may come in contact with the discharged air, the most dependable system of air purification must be used. Decker et al^{9/} reported on the use of a Trent electric incinerator; however, this equipment was expensive to install, required a long retention tube, and malfunctioned when operated at low flow rates. In addition, when the Trent sterilizer required repair, the entire unit had to be removed and returned to the manufacturer. Gremillion et al^{10/} have reported on the use of electric

incinerators, which are less complex than the Trent and are worthy of consideration for certain installations.

E. ULTRAVIOLET AIR STERILIZERS

Ultraviolet (UV) air sterilizers have been reported as being effective^{11/} for inactivating organisms in an air stream; however, they have the distinct disadvantage that the UV lamps must be cleaned and tested frequently. Furthermore, UV has limited penetrating ability, and those organisms protected by dust may not be killed. Therefore, UV treatment of air is probably most useful against droplet nuclei and of less value against dust carrying organisms. Maintenance requirements and operation monitoring are even more severe and critical for UV installations than for electrostatic systems. It is recommended that considerable study be given to the availability of proper monitoring before UV lamps are installed for routine bacteria elimination.

III. FILTER EVALUATION METHODS

A. NONBACTERIAL EVALUATION

Filter material and filters are evaluated on the basis of their ability to remove material from an air stream. Some factors usually considered in testing a filter medium are: resistance at various flow rates, strength and diameter of fibers, rate of clogging or breakdown (temperature and/or resistance), and the minimum sized particle that the filter is capable of arresting. Methods for determining penetration of nonviable material are briefly described below, although they are not necessarily suitable for evaluating filters for arresting bacterial particles.

The National Bureau of Standards (NBS)^{12/} dust spot or blackness test consists of challenging the test filter with a standard dust and drawing samples of the unfiltered and the filtered air through filter papers simultaneously. The air flow and filter areas are adjusted so that the spots on the filter paper are of equal blackness. The ratio of the area of the black spots and air volumes sampled then indicate the effectiveness of the filter. Industrial filters are tested by injecting fly ash from a Cottrell precipitator into the air entering the filter to provide the challenging particles. Electrostatic precipitators and the more efficient types of fibrous filters are commonly tested by using atmospheric dust as a test material.

Methylene blue^{13/} and other dyes have been used to test filters. In these tests, a solution of the dye is atomized into the influent air stream and samples are collected on paper from each side of the filter so that the color density can be compared, thus indicating the degree of penetration.

A method developed by the American Society of Heating, Refrigeration, and Air Conditioning Engineers (ASHRAE)^{14/} compares the weights of dust contained in equal sample volumes of influent and effluent air. This method can be used for testing any filter if the dust chosen is of the proper weight, particle size, and shape. The Air Filter Institute (AFI) test procedure^{15/} is similar to that of the ASHRAE but is more sensitive because it specifies a standard test dust available from a commercial source.

Filter test methods employing smoke generated from different materials have been developed. The concentration of the smoke in the challenging and filtered air is determined by optical instruments, which measure the amount of light scattered by the smoke particles.

A method now used by many agencies for testing filters or filter media is known as the dioctyl-phthalate (DOP) test.^{16/} Smoke is produced in a special generator by heating the dioctyl-phthalate and then mixing it with humidified air. This smoke, when mixed with the proper amount of diluting air, contains particles of 0.3-micron diameter, at a concentration of approximately 40 grains per cubic foot. The smoke is passed through the test filter at its rated velocity, and the concentration of DOP particles in the filtered and unfiltered air is measured by passing an air sample through a smoke penetrometer. This is a photoelectric instrument that measures the amount of light scattered by the passing DOP particles. An accuracy of 0.001 per cent penetration is attained by this method of testing. This test method is one of the least destructive from a loading aspect when evaluating an assembled filter unit.

Another method of testing filters is through the use of triphenyl phosphate containing radioactive phosphorus. Particles removed by the filter or layers of filter media are assayed with a Geiger counter.

B. BACTERIAL EVALUATION

All of the foregoing methods are used for evaluating filters or filter media designed to remove solid and liquid particles from an air stream. Although they may be used to simulate viable bacterial particles, the true performance of a bacterial filter cannot be correctly determined without the actual use of viable bacteria suspended in air moving through the filter at a rated capacity. Particles created from liquids or mineral solids will have different adhesive properties and may or may not remain on filter fibers as readily as bacterial particles. Some forces that cause deposition of particles may also vary in the type of test material used. The bacterial test provides the most sensitive method of evaluating filters because it quantitates each individual organism that penetrates the filter. No standard procedure has yet been adopted to determine the efficiency of filters in removing biological organisms from an air stream; however, test methods used by the authors and described here have been found to be satisfactory and are realistic, since aerosols of viable organisms are used in the evaluation.

Several papers have been published on Serratia indica and Bacillus subtilis var. niger (Bacillus globigii)^{17/} as organisms that may be used as test agents. The selection of the organism for evaluation may be left to the discretion of the investigator; however, it has been found that

consistent results can best be obtained if a heat-shocked suspension of B. subtilis var. niger spores is used. Spores are more resistant to decay than vegetative bacteria, and can be collected with a simple type of bacterial air sampler, such as the cotton collector.^{18/} If vegetative bacteria are used, such as Serratia indica, a liquid impinger or impaction type of air sampler should be used.^{18/} If naturally occurring microfloras, in clumps or on dust particles, are being studied, an impaction-type air sampler is the sampler of choice. The liquid-impinger type of sampler requires greater care in handling than does the cotton collector. Furthermore, since vegetative bacteria die off much more rapidly in the air stream than do spores, they do not give as reliable an index of filter arrestance. Methods for preparing the suspensions and aerosolizing them and information on collecting and culturing media are given in Appendixes A, B, and C.

The system shown in Figure 6 is a typical test arrangement which permits accurate determination of the bacterial arrestance of filters or filter media when spores are used as the test organism. The same system may be used for evaluating filters with vegetative organisms; however a different type of sampler must be used, since cotton collectors are efficient only for the collection of spores. The system is simple, and can be set up relatively quickly. The bacterial organisms are nebulized into a cloud chamber, where the cloud of bacteria is mixed with air. The aerosol is then drawn into the duct through the filter under evaluation at the rated face velocity and is then exhausted through a blower to the outside. Aerosol samples are taken before and after the filter. If the test filter is

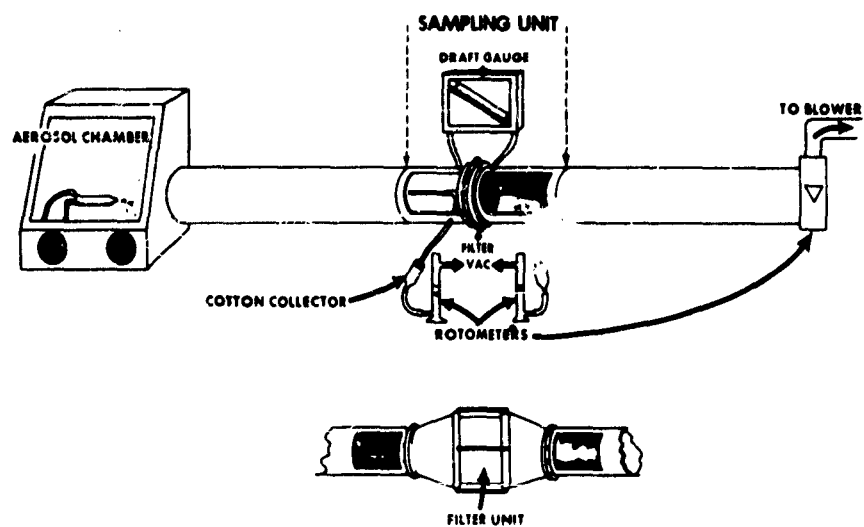


Figure 6. System for Determining Bacterial Arrestance of Filters with Spores.

not the absolute-type, it may be advisable to place an absolute filter in the blower discharge to prevent contaminating the atmosphere with the test bacteria.

The aerosol generator should have a suitable capacity and characteristics so that an aerosol of proper concentration and particle size can be maintained.

C. BACTERIAL EVALUATION OF INSTALLED AIR CLEANING SYSTEMS AND DEVICES

Although most air cleaning devices have been or can be evaluated in the laboratory, where conditions for such studies are favorable, in some instances it may be necessary to measure the cleaning efficiency with the device in place in the ventilation system. This can be done by almost any bacteriology laboratory of moderate size with only a small expenditure for equipment.

If an entire system is to be evaluated, sampling points should be established at each end of the system. However, if only one component is under test, the system should be examined to determine whether a filter, washer, or other cleaning device is interposed between the sampler and the apparatus being evaluated. An attempt to measure the efficiency of an air washer behind a filter in a system probably would show the air approaching the washer to be low in bacterial count. To test a cleaning device, the device must be challenged by a relatively heavy contamination of the approaching air.

Isokinetic sampling of air before and after the filter may be desirable. To accomplish this, the air velocity in the duct should be determined with an anemometer. The size of the sampling probe should be such that the

velocity of the air entering the probe is approximately the same as the velocity of the air in the air duct. The sampling probe should have a gentle curve with a radius of six inches or more. Hose connections should be as short and straight as possible, but where bends cannot be avoided they should have a large radius. Probe, connecting tubing, and sampler should be identical at the pre- and post-sampler locations. The sampler inlet tube can be passed through a cork or rubber stopper which is inserted in a hole in the air duct. Such a connection is satisfactory and permits rapid disconnection of the sampler from the probe.

Two points must be considered if accuracy is to be obtained, (a) the volume of air sampled must be the same at both the inlet and outlet, and (b) care must be taken to insure that both samplers are measuring the same parameter. Methods for accomplishing the former have been detailed elsewhere.^{18/} Error in the latter may occur if samplers (such as the liquid impingement type), which reflect the total number of organisms rather than the total viable particles, are used to determine the efficiency of air cleaning equipment that removes a greater portion of the larger particles and passes the small particles. Clumps of organisms collected upstream by these samplers will be broken up into individual organisms and, if efficiency is computed as described below, the efficiency of the equipment will seem higher than it actually is.

Solid media devices including the slit, sieve, and Andersen samplers most nearly meet the requirements. The Andersen sampler lends itself well to the mechanics of the problem, and in addition to high total collection

efficiency, it gives a rough guide to particle size. Only if the incoming air carries particles of nearly uniform size may a sampler be used which is sensitive to only that particular particle size.

Prior to collection by the samplers, allow several air changes through the air cleaner to assure uniform distribution of particles. Simultaneous samples from the pre- and post-cleaner ports should then be taken. When all preparations have been made, both pumps and samplers should be started at the same time and run for the same time. Do not attempt to rely on one sampler used at both the inlet and outlet successively. Such large variations in bacterial concentration occur at different times that data of this sort are meaningless.

To calculate the efficiency (per cent removal), it is necessary to know the number of viable particles entering the cleaner and the number leaving it.

$$\text{Then efficiency} = \frac{\text{number in} - \text{number out}}{\text{number in}} \times 100.$$

1. Tests with Naturally Occurring Microfloras

An air cleaning device may be tested under the conditions in which it normally operates, including such factors as species of organisms, particle size, concentration, temperature, and humidity; the results will be representative of the situation studied. But conditions such as particle size may change, resulting in a change in cleaning efficiency from that indicated by the test. Within the limitations of the method, however, the evaluation of the cleaning efficiency of a ventilation system while installed can give very useful information.

Air systems using 100 per cent fresh air usually present difficult evaluation problems because the incoming air may carry little contamination. In such situations a sufficient number of replicates must be run to give total upstream and downstream colony counts from all runs of at least 1500. Total counts of this magnitude will give reasonable accuracy of results.

It should be realized, however, that when naturally occurring dust is used to challenge an air cleaner there is no control over size. In most instances, the particles from this source will tend to be large. However, sufficient size distribution is usually obtained if several replicates are run.

If naturally occurring organisms are being used, any good nonselective media may be used. Five per cent blood agar with a heart infusion base is usually satisfactory. The bacteriological procedures are quantitative. No attempt is made to identify the organisms unless the test is designed to determine the efficiency of removal of a specific species.

If some recirculated air is used in the system, dampers should be adjusted or temporary dampers installed to make use of a maximum amount of recirculated air. Then an exhaust vent which leads to the return air duct, should be located near the floor. Even the most lackadaisical sweeping of the floor with a hair broom or shaking dirty linen in front of this exhaust vent will simulate natural contamination and will produce sufficient bacterial contamination in the air to evaluate the efficiency of the air cleaning equipment.

2. Tests with Artificial Microbial Aerosols

Although artificially produced aerosols of Serratia marcescens have been used to test some occupied buildings, this procedure is not recommended in occupied hospitals because debilitated patients should not be subjected to unnecessary contamination. However, coli bacteriophage, which at times has been given in human therapy, is safe. For unoccupied hospitals, Serratia marcescens is an excellent test organism. Bacillus subtilis var. niger is more resistant, and therefore provides a more rigorous test of the facility, but the long persistence of the spore might be undesirable. Extensive experience with this test organism at Fort Detrick has shown that human inhalation of more than one million organisms is not harmful.

To establish the aerosol, a nebulizer or aerosol generator should be placed in the air stream in front of the air cleaner at a distance sufficient to provide a uniform distribution of the aerosol across the face of the device. The recommended minimum distance is eight times the duct diameter. The air sampling probes should be placed in the direction of the air flow and as close as possible to the face of the filter. The location and number of samples taken should be representative of the total volume of air passing through the filter. It is recommended that at least 0.1 per cent of the total volume of air be sampled on each side of the filter.

Whether a naturally occurring or an artificial aerosol is used, the sampling devices and methods should be used as previously described. With the samplers in operation, introduce the bacterial aerosol and attempt to distribute the contamination at a constant low level over the period of the

test, usually ten to fifteen minutes, rather than in one or more heavy bursts. At the end of the sampling period, remove the plates, incubate at the proper temperature and count the colonies when they have grown sufficiently. Calculate the removal efficiency by use of the formula given previously.

D. VIRAL EVALUATION

Perhaps the most important characteristic of aerosols which affects the efficiency of a filter is the particle size distribution. Considerable information is available on filter performance for the micron-sized particles such as bacteria,^{17/} but little is known about the filtration of air-borne viruses.^{19/} Studies on particle deposition and filtration indicate that particles approximately 0.05 to 0.3 micron in diameter are the most difficult to remove from air. Viruses in general are not found in the air as naked, individual cells but are attached to extraneous matter; however, for those organisms that may exist in the submicron sizes, information must be obtained.

All viruses are less than 0.3 micron in diameter and may be 10 millimicrons. One of the chief reasons why there is no information on the efficiency of filters for virus aerosols is the lack of a suitable viable test aerosol. Problems associated with the development of a satisfactory test method include (a) the production of high-titered purified viral suspensions; (b) the development of an aerosol generator for the production of reproducible submicron uniparticulate aerosols; (c) the biological stability of the aerosol, e.g., the effects of temperature and relative humidity; (d) the determination of the most efficient aerosol sampling methods; and (e) the measurement of the size distribution of the aerosol.

Logical candidates for a viral test aerosol are the bacteriophages. The T-3 phage of Escherichia coli has a uniform size of 45 millimicrons ($m\mu$) diameter and is nearly spherical, with a short process or "tail." Phage suspensions used for the production of aerosols must be highly purified, otherwise the aerosol particles will consist mainly of the extraneous cellular materials present in the suspension, with the virus particles contributing little to the mass of the aerosol. Some techniques for virus purification include differential centrifugation, filtration, precipitation with acid, alcohol and salts, ion exchange chromatography, digestion with enzymes, and fractionation in liquid two-phase systems. Aerosol generators such as the Dautrebande, Vaponefrin, the DeVilbiss No. 40 nebulizers, and the University of Chicago Toxicity Laboratory (UCTL) atomizer^{20/} can be used for the production of uniparticulate aerosols with purified phage suspensions by adjusting the viral concentration so that the aerosol droplets contain an average of one virus particle. These atomizers are shown in Figure 7.

Work is in progress at Fort Detrick on the development of a viral aerosol test method. Differential centrifugation has been used to purify and concentrate T-3 phage suspensions. Logical sequence of the work will be the determination of the aerosol particle size distribution, followed by sampling and aerosol viability studies. The final phase of the investigation will be the use of the purified phage in air filtration and purification studies.

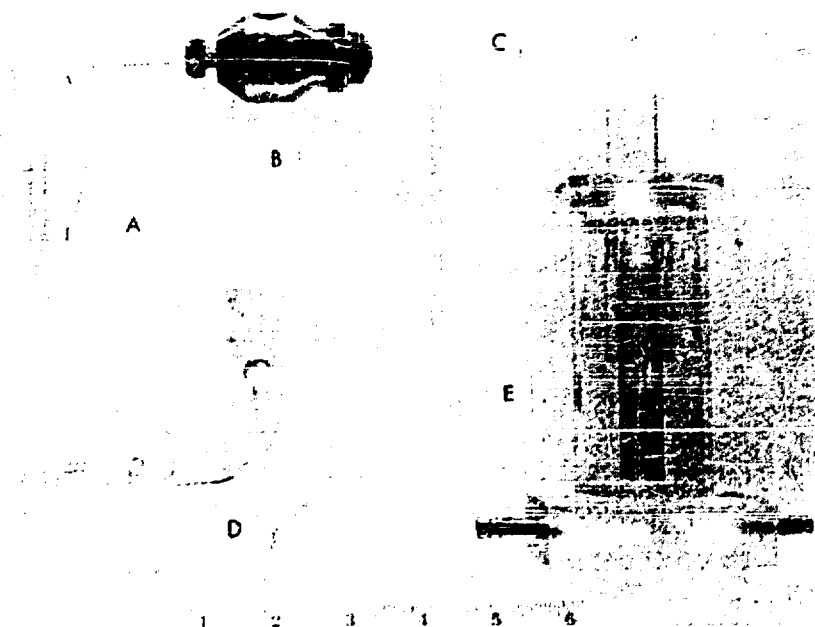


Figure 7. Aerosol Generators.

- A - DeVilbiss Nebulizer
- B - Binks Atomizer
- C - UCTL Atomizer
- D - Vaponefrin Nebulizer
- E - Dautrebande Nebulizer

IV. SELECTION OF FILTERS FOR REMOVING MICROORGANISMS

Factors that should be considered in selecting a filter for a specific installation are: (a) degree of cleaning desired, (b) toxicity or infectivity of particles, (c) concentration and percentage of air-borne particles, (d) volume of air to be cleaned per minute, (e) fire resistance and mechanical strength, (f) time interval between filter cleaning or changes, and (g) cost of installation, operation, and maintenance. The architect, design engineer, or plant engineer must determine which of these factors are important to his particular situation. At times, it may be advisable to measure particle size and dust loading to serve as a basis for estimate of filter life. As previously mentioned, the authors have categorized filters for removing bacteria into four groups: (a) roughing or low-efficiency, (b) medium-efficiency, (c) high-efficiency, and (d) absolute filters (Figures 2 through 5).

In general, the filter that removes the minimum necessary amount of contamination will be selected, since total cost is directly proportional to the filtration efficiency.^{21/} When it is necessary to remove all bacteria from the air, as is desirable in certain hospital areas and industrial situations, or when it is necessary to completely remove specific organisms from the air, such as the exhaust air from a bacteriological research laboratory where considerable concentrations of pathogenic bacteria may be present, one of the absolute filters should be selected. These filters are usually made of fiber glass or of cellulose-asbestos.

Total removal of bacteria is not a requirement in many manufacturing processes, laboratories, and hospitals, and in these cases a less costly air filtration system, (less than 100 per cent efficient), can be used. The high-efficiency (90 to 99 per cent) filters may be satisfactory for such uses. Some manufacturing processes require only medium-efficiency filters (60 to 90 per cent efficient). In the hospital field, present United States practice in operating rooms, maternity wards, nurseries, and other areas is to use cleaned incoming fresh air to dilute the contaminated air of the room. Turbulent mixing of incoming with room air is normal practice. Biological contamination of the air within the room occurs as long as it is occupied by humans or inanimate reservoirs of contamination. Thus contamination in the incoming air is not the only source of contamination, and as long as bacterial aerosols are being generated within the room^{22/} the air can never be entirely free of bacteria regardless of the purity of the entering air. Surgical techniques of scrubbing, gowning, and the use of special types of face masks^{23/} which filter the organisms on both the inhalation and exhalation cycles will do much to reduce the direct human contamination of the room, but the incoming clean air only dilutes the aerosol, which in time reaches a point of equilibrium or constant concentration depending on the cleanliness and volume of incoming air and the rate of aerosol generation. A mathematical study (Section V, B) of this condition shows that there is little difference in equilibrium concentration, whether filters of 90 per cent or 99.99+ per cent efficiency are used. When the turbulent mixing of air is involved it is more practical to use the less efficient filter.

Less frequently found in the United States are ventilation systems designed to displace contaminated air with clean entering air without turbulent mixing. Multiple ceiling inlets and low side wall or baseboard-type exhausts with low velocities are used in an attempt to produce streamlined flow. Thus any point in the room is being washed by air from above, and the contamination of the air at any elevation will depend only on the contamination introduced into the air above that point. In such systems the cleanest entering air is desirable. When critical areas such as operating rooms are involved, a high-efficiency filter to produce the clean air should be used in connection with displacement distribution. Completely nonturbulent displacement is, however, difficult to maintain, and relatively high-velocity streams of sterile air may have to be used in place of low-velocity displacement.

Roughing filters having an efficiency ranging from 10 to 60 per cent usually are all that is required for most air cleaning needs where there is no need to remove all the bacteria, pollen, and dust. Roughing filters are also required as prefilters to prevent loading of the higher-efficiency filters. One type of prefilter, first reported by Silverman and First,^{24/} uses the unique principle of expanding the filter medium as the loading increases. This extends the useful life of the filter to two or three times that of the fixed filters. Bacterial filters of low efficiency are recommended only where there is need to prevent the accumulation of dust and lint in rooms equipped with air conditioners and ventilators.

The dust-loading capacity of any filter depends upon the weight of dirt per unit area that the filter can carry before the resistance across the

filter reaches the point where power consumption is excessive or the volume of filtered air is too low. The time required for a filter to become loaded depends upon the concentration of particles in the air and the volume of air being cleaned. Maintenance costs will depend on the time between necessary filter changes or cleaning and the cost of replacement media or labor for cleaning nonautomatic filters.

Some commercial filters have been evaluated for various applications at government installations. Tables have been prepared placing filters evaluated in one of four categories (Tables II through V). Factors such as maximum operating temperature, fire resistance, moisture resistance, and (at times) chemical resistance must be considered in selecting a filter for a particular situation. Manufacturers of filters will furnish this information upon request. The filters evaluated and listed in this report constitute only a small number of the filters that are commercially available. There are other filters that perform as well as the ones listed, and inclusion of any particular filter does not indicate indorsement of the product by the United States Government or by the authors.

TABLE II. ROUGHING FILTERS
Particle Retention^a/ 10 to 60 Per Cent

NOMENCLATURE	MANUFACTURER	DESCRIPTION
AAF Type HV 2	American Air Filter Co. Louisville, Kentucky	Adhesive-coated, V-crimped wire screen mesh
AAF Type G Media in PL24 Frame	American Air Filter Co. Louisville, Kentucky	Banded glass filaments
AAF Type Airmat 12 Media in PL24 Frame		
Goodyear Pliotron	Goodyear Tire & Rubber Co. Akron, Ohio	Shredded strands of polyethylene medium
Technical Hair Filter	Technical Filter Co. Chicago, Illinois	Animal hair, adhesive-coated
Farr High/Low Velocity Type F/S	Farr Company Los Angeles, California	Adhesive-coated wire mesh screen
Farr 44	Farr Company Los Angeles, California	Adhesive-coated wire 14-mesh screen
Farr 44-68	Farr Company Los Angeles, California	Combination of one section of 2- inch 14-mesh screen followed by one section of 2-inch, 18- mesh screen
Drico Puffglass	Drico Industrial Corp. Passaic, New Jersey	Pad made of spun glass fibers
Aerosolve 35	Cambridge Filter Corp. Syracuse, New York	Glass fibers
Microtron	Microtron Corp. Charlotte, North Carolina	Polyethylene fibers

a. One to five microns.

TABLE III. MEDIUM-EFFICIENCY FILTERS
Particle Retention^a/ 60 to 90 Per Cent

NOMENCLATURE	MANUFACTURER	MEDIUM	CAPACITIES AVAILABLE, cfm	FACE VELOCITY, ft/min	PRESSURE DROP, inches of H ₂ O	OPERATING TEMPERATURE MAXIMUM, °F ^b
AAF Type 25 FG in PL 24 Frame	American Air Filter Co. Louisville, Ky.	Fiber glass	up to 1000	35	0.028	700
AAF Type 100 FG Deep Bed	American Air Filter Co. Louisville, Ky.	Fiber glass	up to 1000 with 5- pocket frame	20	0.25	700
Expandure	Flanders Filters, Inc. Riverhead, N.Y.	Fiber glass	up to 1000	6	0.45	200
FP-2	Farr Company Los Angeles, Calif.	Cotton fabric	1000	32	0.045	Nonflammable
Aerosolve 85	Cambridge Filter Corp. Syracuse, N.Y.	Glass fiber	1000	23	0.22	Fire- resistant

- a. One to five microns.
b. Manufacturer's claim.

TABLE IV. HIGH-EFFICIENCY FILTERS
Particle Retention^a/ 90 to 99 Per Cent

NOMENCLATURE	MANUFACTURER	MEDIUM	CAPACITIES AVAILABLE, cfm	FACE VELOCITY, ft/min	PRESSURE DROP, inches of H ₂ O	OPERATING TEMPERATURE MAXIMUM, °F ^b
Multi-Pak 50 FG	American Air Filter Louisville, Ky.	Glass	700-1000	24	0.42	300
Deep Bed 50 FG	American Air Filter Louisville, Ky.	Glass	1000	20	0.42	700
Micretain	Cambridge Filter Corp. Syracuse 1, N.Y.	Glass- Asbestos	50-1000	5	0.4	220
Aerosolve 95	Cambridge Filter Corp. Syracuse 1, N.Y.	Glass Fiber	500-1800	22	0.35- 0.45	225

a. One to five microns.

b. Manufacturer's claim.

TABLE V. ABSOLUTE FILTERS
Particle Retention^a/ More Than 99.99 Per Cent

NOMENCLATURE	MANUFACTURER	MEDIUM	CAPACITIES AVAILABLE, cfm	FACE VELOCITY, ft/min	PRESSURE DROP, inches of H ₂ O	OPERATING TEMPERATURE MAXIMUM, °F ^b
Type F	American Air Filter Louisville, Ky.	Glass	30-1625	6.	1.0	Up to 1000
Type F	American Air Filter Louisville, Ky.	Ceramic- Asbestos	30-1625	5	1.0	Up to 1600
Ultra-Aire	Mine Safety Appliance Pittsburgh 8, Pa.	Glass	35-1000	5	0.9	Up to 500
Absolute	Cambridge Filter Corp. Syracuse 1, N.Y.	Glass- Asbestos	30-1375	5.5	1.0	220 to 800
Airpure Absolute	Flanders Filters, Inc. Riverhead, N.Y.	Glass F600	30-1625	6.0	1.0	250 to 100
Airpure Absolute	Flanders Filters, Inc. Riverhead, N.Y.	Ceramic- Asbestos	50-1000	5.0	1.0	1600
Magnamedia	Farr Company Los Angeles, Calif.	Glass	30-1625	5.0	1.0	Up to 1000

- a. One to five microns.
b. Manufacturer's claim.

V. INSTALLATION SUGGESTIONS

A. GENERAL CONSIDERATIONS

The installation of an efficient biological air cleaning system to serve certain areas of hospitals, research installations, industrial plants, and civil defense shelters does not in itself necessarily ensure freedom from biological contamination. To maintain the atmosphere of one or more rooms at a low level of bacterial contamination, it is necessary to establish a system of differential pressurization within the building. Clean air flowing into a room, for example a surgical room, must be used for the purpose intended, namely to provide, to the greatest extent possible, a germ-free atmosphere for patients. Use of a pressurized air system permits a minimum interchange of air from areas such as corridors and work rooms, or from other parts of the hospital where the concentration of bacteria will be higher than that normally found in operating rooms. The pressure differential between the surgery rooms and the hallway adjoining it should be from 0.1 to 0.3 inch (water column) with the surgery room having the higher pressure. To facilitate such a balance the use of a cubicle or air lock as shown in Figure 8 is recommended. Pressurizing the air in a space is not enough to prevent movement of contaminated air through a single open doorway against the pressure if there are temperature differentials from floor to ceiling. If one is entering or leaving the pressurized area through an air lock, the first door should be closed before the second door is opened. The use of mechanical or electrical interlocks to prevent doors being opened at the same time should be considered for doors

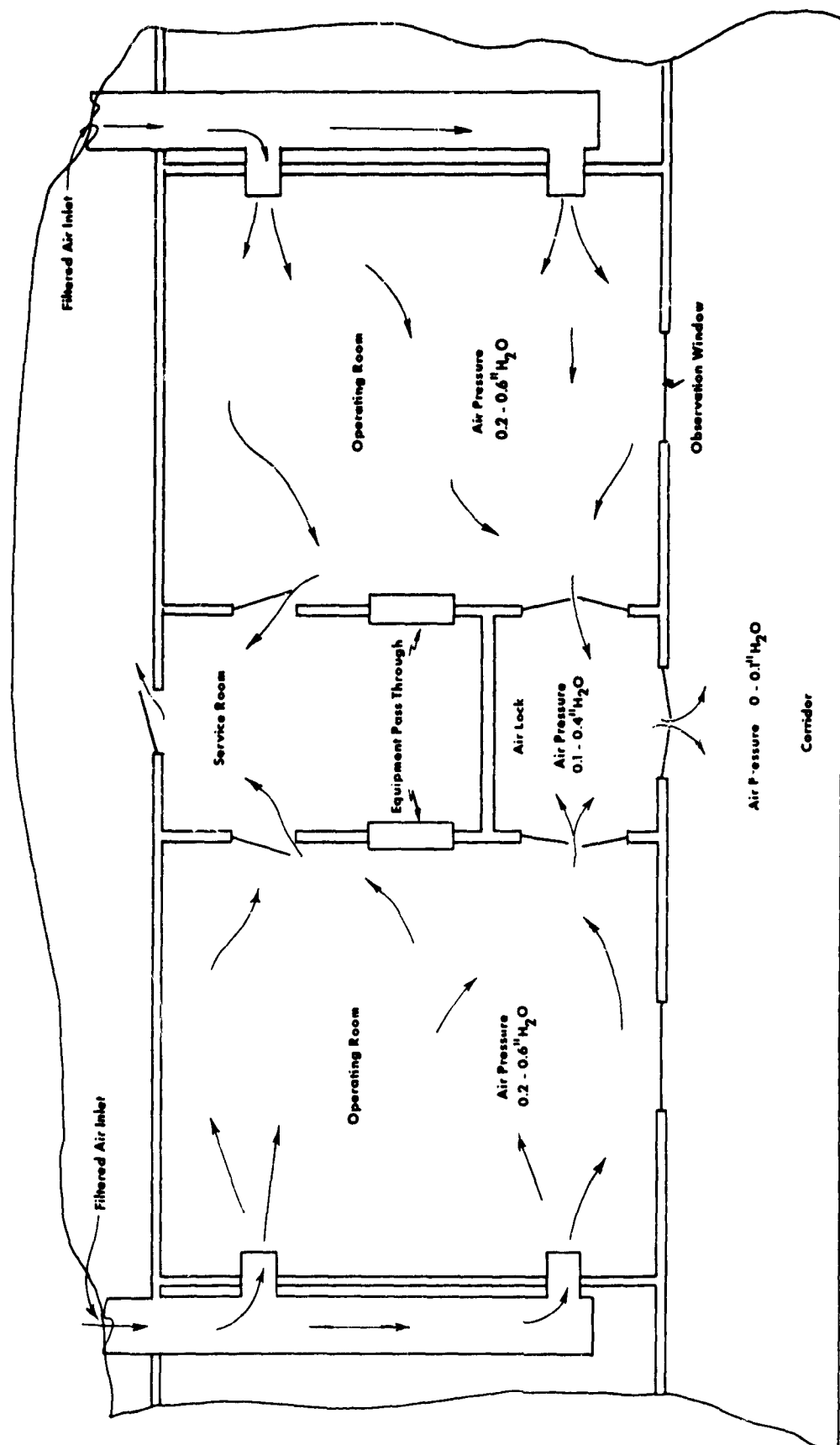


Figure 8. Air Lock to Reduce Infiltration of Contamination.

that are frequently left open. Such an arrangement prevents a sudden drop of pressure and is the best safeguard against flow of contaminated air into clean areas. As an added precaution, it is good policy to provide a downward wash of clean air within the air lock from the ceiling through the floor to remove easily dislodged contaminated particles from the clothing of persons passing through the air lock.

Now let us assume that one is working in a bacteriological laboratory handling considerable quantities of pathogenic microorganisms. In such a situation, it is necessary that the laboratory be under a reduced air pressure,^{25/} so that the laboratory air, which may contain pathogens, is discharged through an efficient exhaust cleaning or incineration system and not permitted to enter hallways or areas where personnel can come in contact with the organisms (Figure 9). If there are several rooms in which pathogenic bacteria are handled, then increasing degrees of reduced pressures should be provided, the more hazardous rooms having greater reduced pressure in relation to the less hazardous areas. In this manner the direction of air flow will always be toward the more dangerous room.

Air supplied to shelters, such as an air raid shelter, will probably be filtered; however, contamination may enter a room or shelter through windows, cracks, or any small openings. Sealing of all unnecessary openings, where practical, is recommended. Contamination will be kept to a minimum if the inside air is maintained at a higher pressure than the outside air. Air required for normal ventilation may provide the necessary pressure (0.1 to 0.6 inch of water); however, if it is not sufficient, the additional sealing of air locks or an increase in air supply by means of higher powered or additional blowers will be required. In areas where it is

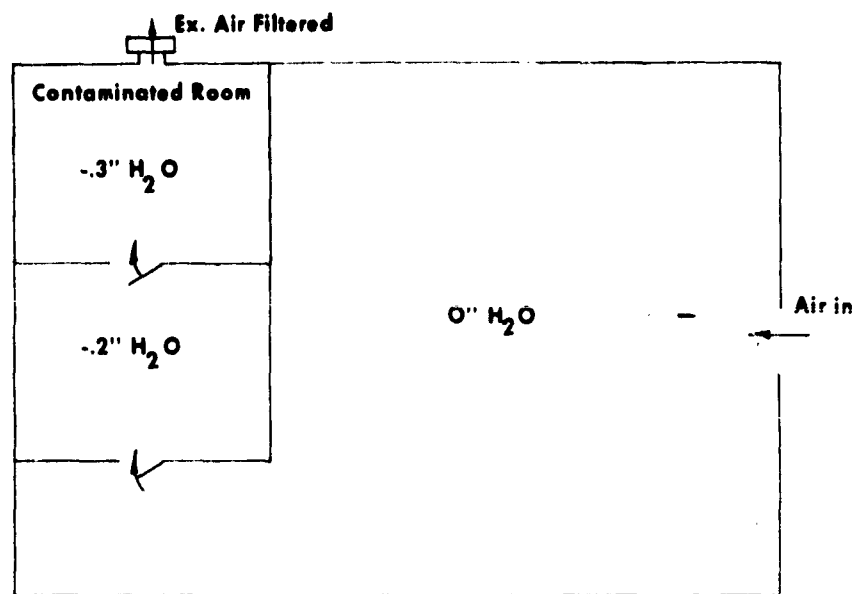


Figure 9. Pressurized System for Bacteriological Laboratory.

critical that the air remain clean and free from microorganisms at all times, the need will justify the additional cost of pressurization.

If an air cleaning system is to operate efficiently, it must be properly installed. A small leak which could allow contaminated air to bypass the filter will negate the effect of the best air cleaner as well as that of the less efficient cleaner. If particulate-free air is required, the seal between the filter and its supporting frame must be gasketed and an adhesive applied, or any other procedure which renders an air-tight seal can be used. Ducts carrying contaminated air under pressure must be air-tight if they are within the protected room.

In designing a filter system it is important that consideration be given to the relative position of the blower and filter as well as the location of the system, i.e., whether it is inside or outside the clean area. The proper method of installing the blower, which will prevent the escape of unfiltered air if there are leaks in the blower, or duct system, is shown in Figure 10. When the air is forced through the filter, any blower leakage will be outward to the contaminated air. When the air is drawn through the filter, leakage in the intake duct will be inward and must pass through the filter.

The installation of a pre-cleaner ahead of high-efficiency and absolute filters is recommended. For this purpose, roughing filters, air scrubbers, and other types of air cleaning equipment can be used. Medium-efficiency filters as pre-filters are more expensive than roughing filters and, except for some automatic types, cannot be cleaned or rejuvenated and must be replaced when they become loaded with dust and other contaminants.

The use of a pre-cleaner increases the useful life of the

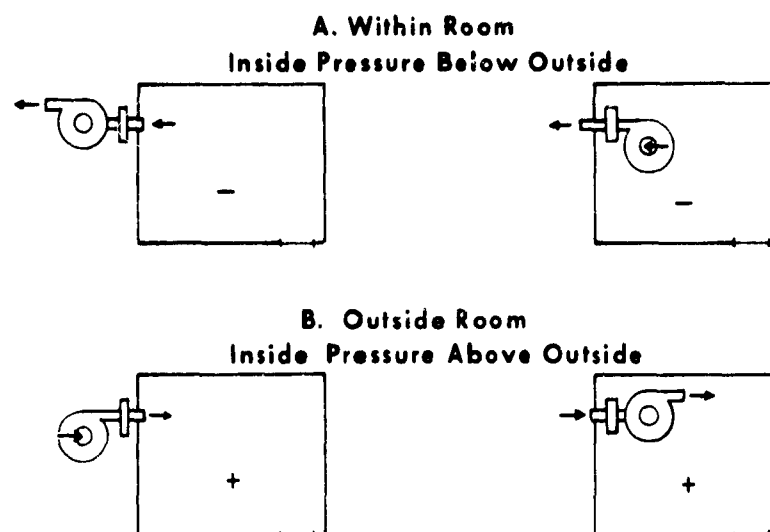


Figure 10. Relative Position of Filter and Blower to Confine Contamination Inside and Outside Room.

high-efficiency and absolute filters many-fold, since it removes gross contamination. The addition of a pre-cleaner does not increase the power cost greatly, as air-flow resistance is usually less than 0.1 inch of water. Consideration should also be given to whether fire resistance of the filter unit is required. In the Atomic Energy program fire-resistive construction of the filter unit is one of the chief requirements. Only under extenuating circumstances are cellulose-asbestos media used (i.e., when hydrofluoric acid, which attacks glass, is present).

Several manufacturers of absolute or high-efficiency filters recommend that filters be changed when the pressure exceeds four inches of water, since filter replacement is generally more economical than the cost of power to overcome additional air-flow resistance. Filter replacement may be determined at a pressure drop selected on a basis of required ventilation and fan capacity of the installation. A draft gauge should be installed in a filter system, with tubing leading to each filter, so that the pressure drop across each filter can be measured. The resistance of the filters should be checked regularly to detect loading before it becomes excessive. A maximum allowable pressure drop is usually established for each type of filter.

In installations where a continuous supply of particulate-free air is necessary, such as civil defense shelters, an auxiliary filter system is desirable in case of unit failure or during replacement. In such installations, an emergency power supply should also be available.

B. AIR FILTRATION REQUIREMENTS FOR HOSPITALS

A problem may be posed as to the importance of efficient air purification systems for hospitals. The contamination of concern in hospitals is mainly produced in the room itself, rather than brought into the room from the outside. In most cases, outside air does not contain many viable organisms, and except in unusual situations only a small percentage of these are pathogens. The exception to this statement is the presence of the spores of Clostridium perfringens and possibly Clostridium titani in many locations. Although the number of spores per cubic foot is low, the large volume of air moving through the system may accumulate a considerable number of organisms on the horizontal surfaces in the spaces served. This possibility should be reduced to the minimum by providing an air cleaning system of high efficiency to clean any air supplied to sensitive areas such as operating rooms and central supply services. Hospital personnel and patients have, on the other hand, frequently been shown to carry pathogenic organisms in their respiratory tracts and on their skin and clothing. The same organism is all too frequently found on the floor and other surfaces of rooms where movement within the room can transfer it to the air. Staphylococci are frequently released into the air from humans.^{26/} E. coli and tubercle bacilli are also released to the atmosphere from humans and are of considerable danger, as has been shown by the classic work of Riley and Wells.^{27/} Thus, there is reason to believe that the dangerous bacterial aerosols are those being generated continuously by humans in the environment.

Building ventilation systems remove this aerosol by (a) streamlined flow across the entire cross section of the room, or (b) diluting the room

air by a turbulent flow of incoming air. Present United States practice uses the latter method almost exclusively.

In the first case, the contamination is washed away from the source by clean air and moved to the exhaust in approximately the concentration in which it is generated. In the second case, the contamination builds up in the turbulent atmosphere in proportion to the rate of generation, the location and rate of exhaust, and the degree of contamination of the incoming air.

For streamlined flow through the room, the cleaner the entering air, the cleaner will be the total room air. In the case of turbulent flow, it is immediately obvious that even if all fresh and recirculated air entering the room is sterile, this sterile air mixing with the room air in turbulent flow merely dilutes the aerosol being generated and does not assure a sterile environment. If ventilation and generation of the aerosol within the room remain constant, the concentration of the organisms in the air will, in time, reach an equilibrium or steady concentration. The exact value of this concentration will depend, of course, upon the rates at which the aerosol is being produced, the methods and velocities by which the diluting air is supplied to the room, and the exhaust locations and velocities by which the mixture is removed.

This raises the question as to whether it is necessary that every organism be removed from the recirculated air in the turbulent hospital ventilation systems found most commonly in the United States, or if one need only remove a sizeable fraction of these organisms. This is capable

of mathematical analysis, and a simple model, containing what is hoped are practical parameters, has been developed.* This solution is in general terms, and the formula (Appendix D) can be used to make calculations for any type of situation, by substituting values for room size, ventilation rate, filter efficiency, etc. It is applicable for all situations where an aerosol is being generated within a closed space and is being continuously removed by filtration or by dilution with pure air.

The model assumed a typical situation that might occur in a hospital room. The following parameters are given:

Assume a room of approximately 5,000 cubic feet (20 by 20 by 12 feet), the air of which is clean at the start. Assume that there are ten air changes per hour and that the air is filtered during each change (Table VI). For this model, 100 per cent recirculation is assumed without the introduction of outside air. The filters are assumed to be 30, 60, 90, or 100 per cent efficient. The latter efficiency is the same, of course, as bringing in completely clean air. Complete mixing of the air in the room is assumed.

No data are available on the concentration of organisms one would expect to be released by hospital personnel, either through their respiratory system or by dislodging organisms resting on surfaces. In an effort to approach reasonable parameters for these values, it was assumed that 1000, 10,000, or 100,000 organisms per minute were being generated by the humans in the room. This range of values includes those that might be encountered

* Prepared by Mr. Floyd H. Taylor, Fort Detrick, Maryland.

TABLE VI. ROOM CONTAMINATION IN ORGANISMS PER CUBIC FOOT
AT END OF ONE HOUR AND AT STEADY STATE

FILTER EFFICIENCY, %	ORGANISMS BEING GENERATED PER MINUTE		
	1,000	10,000	100,000
30	0.0047427* (0.0049915)*	0.047427 (0.049915)	0.47427 (0.49915)
60	0.0002489 (0.0002495)	0.002489 (0.002495)	0.02489 (0.02495)
90	0.0001663 (0.0001663)	0.001663 (0.001663)	0.01663 (0.01663)
100	0.0001497 (0.0001497)	0.001497 (0.001497)	0.01497 (0.01497)

Assumptions: 5,000 cubic feet in room; clean at start. Then air changes 10 times per hour through filters. Complete mixing obtained at all times.

* First figure in the body of the table gives concentration in organisms per cubic foot reached at end of one hour. The second figure, in parentheses, gives the equilibrium or steady state concentration. For development of the mathematical solution of this problem, see Appendix D.

in inhabited rooms, including hospital wards or operating rooms. Higher values would be encountered only in areas where some special activity was carried out, such as the handling of soiled linen.^{28,29/}

In the solution to the problem the value given to contamination rates has no effect on the choice of filters, because the equilibrium concentration reached is directly proportional to the contamination rate for any particular filter efficiency. It also is interesting to note that with these parameters the steady or equilibrium state is essentially reached within the first hour and reached a little more rapidly with the more efficient filters. Table VI shows that the concentration figure (equilibrium) for a 60 per cent efficient filter is 0.0002495, for a 90 per cent filter 0.0001663, and for a 100 per cent filter 0.0001497. There is a considerable reduction in organism concentration between 60 and 90 per cent efficient filters, but comparatively little between the 90 and 100 per cent ones. Thus there is considerable benefit in using a relatively efficient (90 per cent) filter as opposed to a relatively inefficient (60 per cent) filter, but very little additional benefit is gained by using a filter which is essentially perfect, or by supplying completely clean air from an extramural source if distribution in the room results in turbulence. However, if flow in the room is streamlined^{22/} (an almost impossible condition to obtain) sterile inlet air will permit the cleanest possible atmosphere. The nearest practical approach to such distribution and to clean air from high-efficiency filters or equivalent devices should be seriously considered for critical areas.

VI. DECONTAMINATION OF FILTERS

Laboratory buildings in which work is carried out with infectious disease microorganisms should be equipped with bacteriological filters for filtering the air exhausted from the building. These filters must be decontaminated in place or replaced by personnel wearing efficient respiratory protective devices to avoid inhaling secondary aerosols containing pathogenic organisms created when filters are removed from the air system. Protective clothing should be worn to avoid possible contact of the body with pathogens and to avoid transfer of pathogens to clean areas. Filters must be changed when the resistance to air flow increases until an insufficient supply of air is being drawn through the filter system. Also, filters may have to be decontaminated when workmen must enter a potentially contaminated filter system to repair the blower or duct work, or to change a ruptured filter. There are several methods by which these filter systems can be decontaminated. The choice of method depends upon the system and facilities available. A filter unit or complete system may be decontaminated by chemicals or by heat.

A. METHODS OF DECONTAMINATION

1. Formaldehyde

Formaldehyde can be used to sterilize installed filters when air is exhausted to the outside. Recirculating air systems can be decontaminated with formaldehyde if personnel are not present in the building. Also, experimental animals must be removed from research laboratories before decontamination. The bactericidal efficiency of formaldehyde vapor is a direct

function of the concentration, relative humidity, and temperature. A temperature of 75°F or higher is desirable, and the effectiveness of the disinfectant decreases rapidly as the relative humidity drops below 70 per cent. When a filter or filter system is decontaminated the air flow should be reduced to a minimum so that a high vapor concentration can be maintained.

Most buildings are equipped with a heating system by which the temperatures can be maintained at 75°F or higher. The relative humidity can be increased by spraying water from a vaporizer or by the same device used to disseminate the disinfectant. Even though the humidity has been raised, it is often necessary to continue to spray water or inject steam at a reduced rate to maintain a high relative humidity. Almost any method of disseminating formaldehyde into an air duct in suitable quantities is satisfactory when using this chemical as a filter decontaminant. Steam ejectors, steam vaporizers, or other types of atomizers can be used, or a steam line equipped with a steam ejector can be permanently installed in the system. For treating large systems, a large-capacity mechanical-type vaporizer can be used. Some of the disseminators that have been found satisfactory for vaporizing formaldehyde are shown in Figure 11 and listed in Table VII.

Formaldehyde has the disadvantage of polymerizing on surfaces. The polymers are rather difficult to remove, but polymerization can be partially avoided by using a dilution of standard formalin solution (37 per cent HCHO) with methanol (five parts formalin solution to three parts methanol). Either the formalin solution or the formalin methanol mixture may be used for any of the applications described. Formaldehyde solution should be disseminated into the filter plenum at one milliliter per minute for each cubic

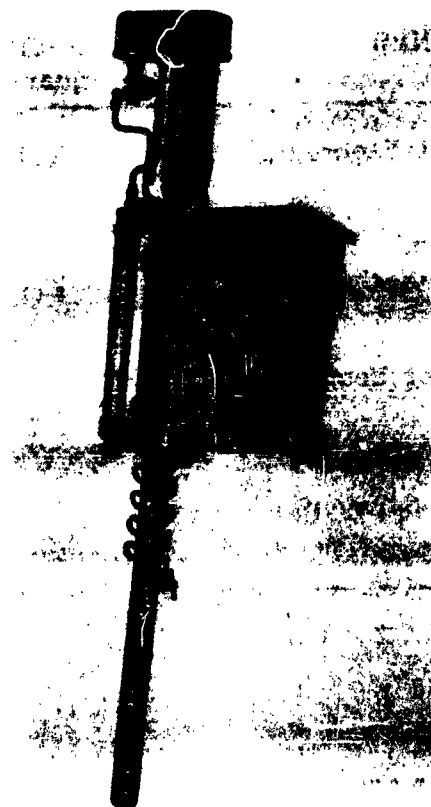
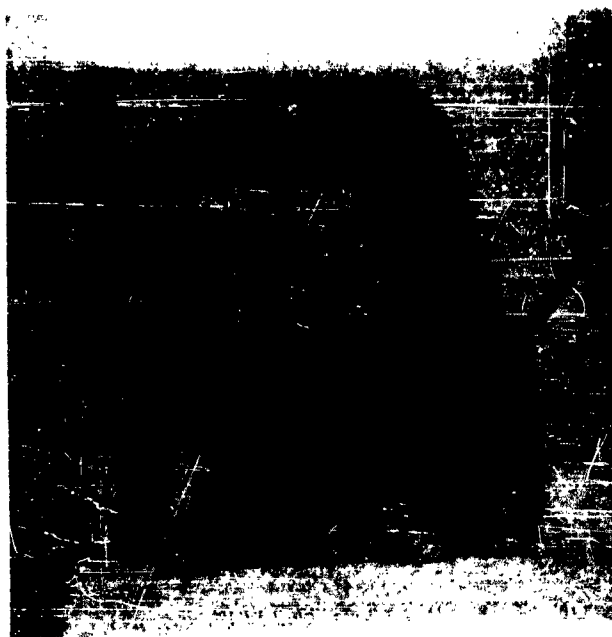


Figure 11. Disseminators for Decontaminants.

foot of air flow for 30 minutes, e.g., if the air flow is 600 cubic feet per minute, then 18,000 milliliters of formaldehyde solution ($600 \times 1 \times 30$) will be disseminated in 30 minutes. When decontaminating an air duct, wind direction is important. If the wind direction is from the air exhaust stack to the supply grill, then formaldehyde will be drawn into the building. This condition must be avoided.

After decontamination, the filters are generally allowed to aerate overnight, after which they may be removed and discarded with minimum precautions.

TABLE VII. COMMERCIAL SOURCES OF SOME SPRAYERS SUITABLE
AS FORMALDEHYDE DISSEMINATORS

NAME	CONTAINER CAPACITY, quarts	MANUFACTURER
Hydromist Vaporizer	1.4	Arnold Laboratories 7103 Laurel Canyon Blvd. North Hollywood, California
Microsol Mechanical Aerosol Generator	2	Silver Creek Precision Corp. Silver Creek, New York
Challenger Mechanical Sprayer	4	Z & W Manufacturing Corp. 30240 Cleveland Blvd. Wickliffe, Ohio
Dyna-Fog Sprayer	8	Curtis Automatic Devices, Inc. Westfield, Indiana

2. Ethylene Oxide

Another decontaminant that may be employed is ethylene oxide; however, its use is limited to gas-tight enclosures because the chemical is highly penetrating. To eliminate the hazard of the flammability of ethylene oxide, it is mixed with chlorofluorohydrocarbons or carbon dioxide. The filter system must be divided by guillotine dampers so that the gas may be admitted to an air-tight area. This type of a construction may be costly. If the procedures previously discussed are impractical, as a last resort the filter unit can be removed from the ventilation system and placed in a modified autoclave, modified drum, or polyethylene bag to which a mixture of ethylene oxide and carbon dioxide or chlorofluorohydrocarbons (i.e. Freon) is admitted. If this method of decontamination is selected, personnel must be provided with adequate protection to avoid respiratory or body contact with viable pathogenic organisms that might be shaken off the filter while it is being removed from the ventilation system.

3. Self-Sterilizing Filters

Recent studies on the disinfection of hospitals have involved the use of air filters impregnated with a bactericide.^{30/} Tests have been conducted on filters impregnated with bis (tri-n-butyltin) oxide and dialkyl (C8 to C18) dimethyl ammonium chloride. A vegetative Gram-negative bacterium was used as the test organism. Results of these tests indicate that no appreciable reduction in passage of the viable microorganisms occurs when a filter is treated with this type of germicide. In these tests, it was found that the passage of viable organisms decreased in both treated and untreated filters as the relative humidity increased from 70 to 95

per cent, thus both types of filters are more efficient at higher relative humidities. This phenomenon had been noted in previous tests with other types of filter system. No known commercial germicide that has been incorporated into self-sterilizing surface materials or on filters possesses any significant ability to reduce the bacterial count on the filters unless it is surrounded by an environment of extremely high humidity.

4. Heat Sterilization

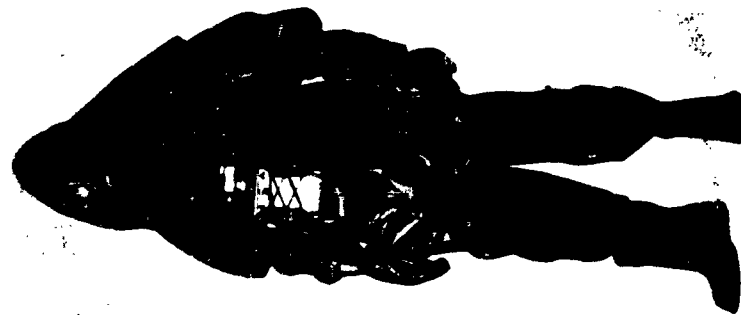
Filters may be sterilized with heat by installing heating elements around noncombustible filters and sealing off the filter units so that the heat will be retained in a fireproof confined area. This method has been used in spun-glass air filter systems that exhaust air from bacteriological safety cabinets. If the filter cannot be decontaminated in place, it can be removed by masked personnel in protective clothing, placed in a bag, and incinerated, or it may be placed in a steam autoclave and decontaminated at 15 psig for 30 minutes.

B. DECONTAMINATION CLOTHING

Personnel who work with contaminated filters must be protected from infection by microorganisms in the filters. This protection includes wearing an efficient respiratory mask and protective clothing (Figure 12) or washable outer clothing such as laboratory overalls. The protective clothing should be removed as soon as possible after potential contamination. It is always advisable to shower and don clean clothing as soon as possible after working in a potentially contaminated area.



NON AIR SUPPLIED



NON AIR SUPPLIED



AIR SUPPLIED

Figure 12. Protective Clothing.

Commercial sources of protective clothing include:

Mine Safety Appliances Company
201 North Braddock Avenue
Pittsburgh 8, Pennsylvania

Snyder Manufacturing Company, Inc.
1458 Fifth Street, N.W.
New Philadelphia, Ohio

Standard Safety Equipment Company
431 North Quenten Road
Palatine, Illinois

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APPENDIXES

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APPENDIX A

PROCEDURE FOR GROWING BACILLUS SUBTILIS VAR. NIGER AND SERRATIA MARCESCENS

Stock cultures of Bacillus subtilis var. niger (B. globigii) and Serratia marcescens can be obtained from the American Type Culture Collection, 2112 M Street, N.W., Washington 7, D.C. Since these bacteria are conventionally regarded as nonpathogenic for man, no special safety precautions are required when working with them. Aseptic laboratory techniques should be used to prevent contamination of the air and laboratory facilities with the test bacteria and to prevent contamination of the test cultures with other organisms. Respiratory protection, in the form of a gas mask or a commercial respirator, should be provided whenever exposure to concentrated aerosols of B. subtilis or S. marcescens is anticipated.

I. BACILLUS SUBTILIS VAR. NIGER

Four tryptose agar slants (Table I) are inoculated from the stock culture and incubated at 34° to 37°C for 24 hours. Following incubation, a heavy, yellow-orange pigmented colony growth will be visible on the agar surface.

Seven 250-ml Erlenmeyer flasks, each containing 50 ml of tryptose broth (Table I) are inoculated with a large loopful of colony growth from the agar slants. These flasks are then incubated on a shaker for eight hours at 34° to 37°C.

After eight hours, a 20-ml inoculum from the Erlenmeyer flasks is added to each of 16 three-liter Fernbach flasks, each containing 250 milliliters of tryptose broth. The Fernbach flasks are then incubated on a shaker for five to six days at 34° to 37°C.

After incubation, the liquid culture is centrifuged until the supernatant is clear. The supernatant is decanted and the cells are resuspended in 100 ml of sterile distilled water and recentrifuged. Repeat this washing and centrifuging procedure three times. The cells are then resuspended in sterile distilled water and transferred to a sterile bottle or flask. This procedure will yield a clean cell suspension containing approximately 90 per cent spores with a concentration of 1×10^9 to 1×10^{10} spores per milliliter.

The spore suspension is heat-shocked by immersing the bottle in a 60° to 65°C water bath for 30 minutes to eliminate all vegetative cells. The 30-minute immersion time begins when the temperature of the spore suspension reaches 60°C. After heat-shocking, the concentration of the spore suspension is determined by the standard pour-plate method, using tenfold serial dilutions plated in triplicate with tryptose agar to obtain countable plates (30 to 300 colonies per plate). When not in use, all spore suspensions should be stored in a refrigerator at 4° to 6°C. At this temperature, the spores will remain viable for years without an appreciable change in concentration.

II. SERRATIA MARCESCENS

Inoculate two tryptose agar slants from the stock culture of S. marcescens and incubate at 30°C for 24 hours. After incubation, a heavy, red-pigmented colony growth will be visible on the agar surface.

Inoculate four 250-ml Erlenmeyer flasks, each containing 50 milliliters of tryptose broth, with a large loopful of colony growth from the agar slants. Incubate these flasks on a shaker for 18 hours at 30°C.

Add a 10-milliliter inoculum from the Erlenmeyer flasks to each of 16 three-liter Fernbach flasks, each containing 250 milliliters of tryptose broth. Incubate the flasks on a shaker for 18 hours at 30°C.

After incubation, the liquid culture is centrifuged until the supernatant is clear. The supernatant is decanted and the cells are suspended in sterile tryptose saline (Table I) and centrifuged. Repeat this washing and centrifuging procedure three times. Resuspend the cells in sterile tryptose saline* and transfer to a sterile bottle or flask. This procedure will yield a clean cell suspension containing approximately 1×10^9 cells per milliliter.

The concentration of the cell suspension is determined by the spread-plate method, using tenfold serial dilutions plated in triplicate on tryptose agar, to obtain countable plates (30 to 300 colonies per plate). All cell suspensions should be stored in a refrigerator at 4° to 6°C when not in use. At this temperature, the cell suspensions will remain suitable for use for approximately 30 days.

* 100 ml.

TABLE I. FORMULAS FOR CULTURE MEDIA

TRYPTOSE AGAR

Bacto-tryptose	2.0%
Dextrose	1.0%
Sodium chloride	0.5%
Agar	2.0%
Water	to 1 liter

- Add ingredients to water and heat to boiling to dissolve.
- Adjust pH to 7.2 to 7.4.
- Dispense into flasks and test tubes and sterilize by autoclaving for 15 minutes at 15 pounds pressure (121°C). Final pH should be 7.0 to 7.2.

The individual ingredients can be obtained from most suppliers of biological media.

TRYPTOSE BROTH

Bacto-tryptose	2.0%
Dextrose	1.0%
Sodium chloride	0.5%
Water	to 1 liter

- Add ingredients to water and heat gently to dissolve.
- Adjust pH to 7.2 to 7.4.
- Dispense into flasks and sterilize by autoclaving for 15 minutes at 15 pounds pressure (121°C).

The individual ingredients can be obtained from most suppliers of biological media.

TRYPTOSE SALINE

Tryptose	0.1%
Sodium chloride	0.5%
Water	to 1 liter

- Add ingredients to water and heat gently to dissolve.
- Adjust pH to 7.2 to 7.4.
- Dispense into flasks and sterilize by autoclaving for 15 minutes at 15 pounds pressure (121°C). Final pH should be 7.0 to 7.2.

The individual ingredients can be obtained from most suppliers of biological media.

APPENDIX B

AEROSOL GENERATION

For accurate assessment of bacterial filter penetration, a challenge aerosol of particles of constant concentration and uniform size must be maintained throughout the test. Ideally, the aerosol should contain bacterial particles which are the size of each individual organism. Filters are evaluated with an aerosol containing particles one to five microns in diameter. The concentration of the challenging aerosol should be maintained at a constant high level, especially when absolute filters are being tested. If filtration efficiency anticipated is greater than 99.99 per cent and only a small volume of the effluent air is collected as a sample, it will not be representative. If a challenge aerosol of very high concentration is used or a large volume of the effluent air is collected in the sample, it will be more representative. It is suggested that the challenge aerosol have a concentration of 1×10^4 to 1×10^6 organisms per liter of air.

The organism selected as a test aerosol should be nonpathogenic, relatively stable in air, easy to produce in high concentrations, and simple to culture and assay. Many tests are performed with a spore-forming organism (B. subtilis var. niger) because it has all these characteristics.

When testing small sections of filter medium or low-capacity filters, small glass or plastic atomizers may be used to spray the suspensions of test organisms (Figure 7 of text and Table I of this Appendix). Examples of these are the University of Chicago Toxicity Laboratories atomizer, the Vaponefrin, and the DeVilbiss No. 40 models. Each of these will discharge

TABLE I. AER. GENERATORS

TYPE	DESCRIPTION	NUMBER	RECOMMENDED SPRAY RATE, ml/min	MANUFACTURER
Binks	Direct-spray peripheral air-jet atomizer	50-100	2-10	Binks Corporation 3114-40 Carroll Avenue Chicago, Ill.
Vaponefrin Nebulizer	Indirect-spray atomizer or nebulizer	Modified for lab use	0.2	Vaponefrin Co. Upper Darby, Pa.
Devilbiss	Indirect spray	40	0.2	Devilbiss Co. Somerset, Pa.
UCTL	All-glass, direct-spray peripheral air-jet atomizer	None	0.2	No Commercial Source
Dautrebande	Indirect spray	None		J. H. Emerson Co. 22 Cottage Park Avenue Cambridge, Mass.
Mechanical Aerosol Fog Generator	Centrifugal spray	202	50-100	Silver Creek Precision Corp. Silver Creek, N. Y.

approximately 0.2 milliliter of suspension per minute with an air requirement of five to six liters per minute at 15 psig. When properly used, all of these produce aerosols containing a high percentage of particles approximately 1 micron in diameter. The final size of the particles depends upon the characteristics of the spraying device, the physical characteristics of the suspension, and the relative humidity of the environment.

The Binks Series 50 (Figure 7 of text) and other similar two-fluid spray nozzles can be used where greater volumes of challenge aerosols are needed. One Binks nozzle is capable of generating sufficient bacterial aerosol to raise 50,000 liters of air per minute to a concentration of 1×10^5 organisms per liter of air.

If still larger volumes of challenging aerosols are required, the centrifugal or spinning disc types may be used, such as the Microsol Mechanical Aerosol Generator (Figure 11 of text). This type of generator can discharge as much as 100 milliliters of suspension per minute; however, it is not as efficient as the smaller generators for producing a high percentage of small particles. All generators should be positioned an adequate distance upstream from the filter to allow liquid droplets to evaporate and reach equilibrium and to allow the larger particles of the challenge aerosol to settle out before a sample is collected.

APPENDIX C

MEDIA FOR COLLECTING AND CULTURING MICROORGANISMS

The use of proper collecting and culturing media is of fundamental importance because the determination of the number of viable microorganisms contained in the sample depends primarily on the enumeration of bacterial colonies developing either directly on the nutrient collecting medium or in subsequent plate cultures of a sample.

Many collecting and culturing media are available. The selection of a nutrient medium will depend primarily on the nutritional requirements of the organism or organisms under study. The collected microorganisms must remain viable without a change in concentration or decay until samples are taken for culture. Two collecting fluids that may be used for vegetative organisms are tryptose saline and buffered gelatin. These media are used also as diluting fluids to obtain suspensions suitable for plating. Buffered saline and buffered water are used only for the collection of spores and other resistant microbial forms.

Two media that may be employed for culturing the liquid samples or the water containing organisms washed from the cotton are tryptose agar and fortified nutrient agar. Surface plating methods are used. A suitable medium for the collection of staphylococcus is one that contains a blood agar base.

The media listed in Table I, as well as others can be prepared either with the dehydrated products from suppliers of biological media or according to formulas in textbooks and manuals on microbiology.

TABLE I. FORMULAS FOR MEDIA

<u>Impinger Fluid</u>	
	Gm/Liter of Distilled Water
<u>Tryptose Saline</u>	
Bacto-tryptose	1
Sodium chloride.	5
Adjust pH to 7.0 ± 0.2	
<u>Buffered Saline</u>	
Sodium chloride.	8.5
Disodium phosphate (anhydrous)	5.8
Potassium dihydrogen phosphate (anhydrous)	3.5
Adjust pH to 7.0 ± 0.2	
<u>Gelatin Diluent</u>	
Bacto-gelatin.	2
Disodium phosphate (anhydrous)	4
Adjust pH to 7.0 ± 0.2	
<u>Buffered Water (Stock)</u>	
Potassium dihydrogen phosphate	34
Adjust pH to 7.2. Add 1 to 2 ml of stock to 1 liter of distilled water.	

Solid Media

<u>Enriched Nutrient Agar</u>	
Bacto-beef extract	3
Bacto-peptone.	10
Yeast extract.	5
Dextrose	10
Agar	20
Adjust pH to 7.0 ± 0.2	
<u>Tryptose Agar</u>	
Bacto-tryptose	20
Dextrose	1
Sodium chloride.	5
Agar	15
Adjust pH to 7.0 ± 0.2	
<u>Blood Agar</u>	
Beef heart, infusion from.	500
Bacto-tryptose	10
Sodium chloride.	5
Agar	15
Adjust pH to 7.0 ± 0.2 . Add 5 per cent fresh defibrinated horse, rabbit, sheep, or beef blood.	

APPENDIX D

MATHEMATICAL MODEL ON HOSPITAL VENTILATION

Let

N = number of organisms/cu ft present at time t in minutes

V = volume of room in cubic feet

K = number of complete changes of room volume/hour

b = total number of organisms/minute entering because of human presence

a = efficiency of the filter.

Then,

$\frac{NKV}{V60} (1-a) \Delta t$ = total number of organisms/cu ft entering during the interval Δt because of the inefficiency of the filter.

$\frac{1}{V} b \Delta t$ = total number of organisms/cu ft entering during interval Δt because of contamination from individuals.

$\frac{1}{V} \frac{NKV}{60} \Delta t$ = total number of organisms/cu ft leaving during interval Δt .

ΔN = (total number of organisms/cu ft entering) - (total number of organisms/cu ft leaving)

$$\Delta N = \frac{NK}{60} (1-a) \Delta t + \frac{b}{V} \Delta t - \frac{NK}{60} \Delta t$$

$$\frac{\Delta N}{\Delta t} = \frac{b}{V} - \frac{KNa}{60}$$

$$\frac{dN}{dt} = \frac{b}{V} - \frac{KaN}{60} = \frac{b}{V} \left(1 - \frac{aKV N}{60b} \right)$$

$$\frac{dN}{1 - \frac{aKV N}{60b}} = \frac{b}{V} dt$$

$$- \frac{60b}{aKV} \int \frac{- \frac{aKV dN}{60b}}{1 - \frac{aKV N}{60b}} = \int \frac{b dt}{V}$$

$$-\frac{60b}{aKV} \ln \left(1 - \frac{aKVN}{60b} \right) = -\frac{b}{V} t + C$$

$$-\frac{60b}{aKV} \ln \left(1 - \frac{aKVN}{60b} \right) = -\frac{b}{V} t$$

$$-\frac{60}{aK} \ln \left(1 - \frac{aKVN}{60b} \right) = t$$

$$\ln \left(1 - \frac{aKVN}{60b} \right) = -\frac{aKT}{60}$$

$$\left(1 - \frac{aKVN}{60b} \right) = \exp \left(-\frac{aKt}{60} \right)$$

$$\frac{60b}{aKV} \left[1 - \exp \left(-\frac{aKt}{60} \right) \right] = N$$